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BIOLOGICAL TREATMENT OF ACID MINE WATER

Franco Rabolini, et al

Syracuse University

Prepared for:

Federal Water Quality Administration

July 1971

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Final Report

Submitted to:

Federal Water Quality Administration
Environmental Protection Agency

by

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Department of Chemical Engineering and Materials Science

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ABSTRACT

The strain ATCC-7577 of Desulfovibrio Desulfuricans was used to test the feasibility of treating acid mine water biologically. Sulfate reduction rates were measured with lactate media at different temperatures, influent pH, and sulfate concentrations in batch and continuous reactors. Among the several additional organic substrates tested, digester sludge appears to be the most attractive because of its low cost and its neutralizing properties. Sulfate reduction in mixtures of acid mine water and digester sludge were demonstrated in a semi-continuous reactor and the sulfate reduction rates were measured.

The results of these studies indicate that the sulfate reduction rate is controlled primarily by the organic substrate concentration, the concentration of sulfate reducing bacteria, the temperature, and pH in the reactor. The sulfate reaction rate appears to be proportional to both the organic substrate and bacteria concentrations and at a pH of 7 and a temperature of 37°C, this rate was observed to be approximately:

$$r = 0.33 \left(\frac{\ell}{gr.hr.}\right) Y_{s/x}\, C \cdot X \ (gm/hr.\ell)$$

Reducing the temperature to 10°C reduced the rate to 1/4 of the rate at 37°C. Reducing the pH also reduced the sulfate reduction rate with no sulfate reduction observed at reactor pH's below 5.5.

This report was submitted in fulfillment of Grant No. WP-01460-01 between the Federal Water Quality Agency (EPA) and Syracuse University.

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1. CONCLUSIONS

A detailed picture of the growth of sulfate-reducing bacteria is given and the feasibility of treating acid mine water biologically with sulfate-reducing bacteria was tested. A great portion of research was carried on with a modified Postgate medium at different temperatures, at different initial or influent pH, in continuous or in batch runs. The result of this analysis should be a standard comparison and a guideline for further studies with natural media. The process has been proved feasible; actual acid mine water can be treated biochemically with sulfate-reducing bacteria.

The sulfate reduction rate in Postgate media was found to be proportional to the product of the organic substrate and bacteria concentrations. At 37°C and a reactor pH of 7, the rate was given approximately by:

$$r\left(\begin{matrix}\text{sulfate} \\ \text{reduction}\end{matrix} \frac{\text{gr.}}{\ell\text{.hr.}}\right) = 0.33 \left(\frac{\ell}{\text{gr.hr.}}\right) Y_{s/x}\, CX$$

The rate at 10°C was observed to be about 1/4 the rate at 37°C. Reducing the pH also reduced the sulfate reduction rate with no growth observed at a reactor pH of 5.5 or below.

The main problem is to find a cheap organic nutrient for D. Desulfuricans; sodium lactate was the most effective substrate, but its cost makes its use impossible for industrial application.

Digester overflow sludge can be used as an organic substrate, although it has a relatively low concentration of organic carbon and a more concentrated source would be desirable. Digester sludge does have the advantage of partially neutralizing the acid mine water as well as providing organic substrate for the bacteria.

2. RECOMMENDATIONS

Further studies investigating digester sludge as an organic substrate for biological sulfate reduction of acid mine water should be made. These should include the effect of sludge/mine water ratios on the reduction rates, the total sulfate reduced, and the effluent pH; and the nature and settling characteristics of the precipitate (probably largely FeS). Other organic nutrients such as sawdust and milk and cheese wastes should be further tested.

In addition, pretreatment of reactants, post-treatment effluents, and separation, handling, disposal, and recovery of by-products and chemicals should be explored.

3. INTRODUCTION

3.1 Coal and Coal Mines

In the last several decades, coal was displaced as an energy source by other fuels such as gas, oil, and uranium (1). In 1900 coal provided 71.4% of all energy requirements, while in 1960 it accounted for only 28.7%. However, the estimated reserves of coal are much larger than any other fuel; therefore, the predicted shortage of other fuels and future technological developments, like fluidized bed boilers (2), could make coal competitive once again.

There are basically two types of coal mines; strip and underground mines. Strip mines and spoil piles have to be drained from rain, while underground mines must be drained from infiltrating water and underground sources. Mine drainage is acidic because of sulfuric acid; moreover, it contains iron, aluminum, calcium and magnesium ions. Table 1 shows the composition of some acid mine water. Every year approximately 500 billion gallons of acid mine water, containing from 5 to 10 million tons of sulfuric acid, pollute 10,000 miles of streams and more than 15,000 acres of impounded waters (3). This water destroys aquatic life and damages cement and iron structures.

3.2 Acid Mine Water Formation

Table 2 shows the composition of different types of coal (4). Sulfur is present as pyrite, which reacts with atmospheric oxygen and water and produces sulfuric acid and iron sulfate.

$$\text{(A)} \qquad FeS_2 + 3.5\ O_2 + H_2O = Fe^{+++} + 2\ SO_4^{--} + 2\ H^+$$

This reaction is similar to the industrial process for the production of sulfuric acid; however, at room temperature the rate of the chemical reaction is negligible. It has been proved that bacteria of the group Ferrobacillus-Thiobacillus can make the reaction proceed at an appreciable rate at low temperatures.

The suggested mechanism is (5):

$$\text{(B)} \qquad FeS_2 + 14\ Fe^{+++} + 8\ H_2O = 15\ Fe^{++} + 2\ SO_4^{--} + 16\ H^+$$

$$\text{(C)} \qquad 14\ Fe^{++} + 3.5\ O_2 + 14\ H^+ = 14\ Fe^{+++} + 7\ H_2O$$

The oxidation of ferrous ion to ferric ion is the controlling step of this reaction and, therefore, it controls the rate of the entire reaction (6).

The reaction (B) cannot occur at significant rates in the environment of mines, unless there is a biological catalysis between a solid, a liquid medium, and a gas; therefore, any kinetic or mass transfer step can be important in determining the rate.

Table 1 - Composition of Acid Mine Water

	pH	Fe^{+++} (ppm)	SO_4^{--} (ppm)	Al^{+++} (ppm)	$Ca^{++} + Mg^{++}$ (ppm)
West Virginia	3.1	90	850	15	-
Bennett	3.2	16	290	30	-
Proctor 1	3.23	10	250	22	-
Proctor 2	2.86	550	1500	135	-
Norton	2.40	2200	9555	348	307

Table 2 - Composition of Coal

	C	H_2	N_2	S	O_2
Anthracite	94.9	1.8	0.7	0.8	1.8
Low Volatile Bitominous	91.4	4.6	1.2	0.7	2.1
Medium Volatile Bitominous	89.5	4.9	1.7	0.4	3.5
High Volatile Bitominous A	85.0	5.7	1.6	0.8	6.9
High Volatile Bitominous B	82.4	5.7	1.5	1.6	8.8
High Volatile Bitominous C	79.2	5.9	1.5	2.9	10.5
Lignite	72.4	5.3	1.1	0.5	15.0

Composition in percentage weight

Moreover, if we consider that bacterial activity must be supported, the availability of nutrients can also be a limiting factor.

Even if it is quite difficult for the aforementioned reasons to study accurately the bacterial activity in the environment of mines, there is some evidence that

- iron oxidizing bacteria can increase the rate of pyrite oxidation by maintaining a high ferric-ferrous ion ratio in the solution surrounding the pyrite
- the rate of oxidation of pyrite is proportional to the concentration of bacteria in the aqueous solution close to the pyrite surface
- bacterial oxidation of pyrite is likely to occur in environments like refuse piles or spoil banks
- the oxygen concentration does not affect the oxidation rate, unless it drops below a critical value
- the water-pyrite ratio is important, because at a low ratio water-pyrite, the reaction creates a pH so low that it is not tolerable for microorganisms.

Once ferrous sulfate is discharged, it can be further oxidized to ferric sulfate,

(D) $4\ FeSO_4 + 2\ O_2 + 2\ H_2SO_4 = 2\ Fe_2\ (SO_4)_3 + 2\ H_2O$

the hydrolysis of ferric sulfate

(E) $Fe_2\ (SO_4)_3 + 6\ H_2O = 2\ Fe\ (OH)_3 + 3\ H_2SO_4$

(F) $Fe_2\ (SO_4)_3 + 2\ H_2O = 2\ Fe\ (OH)\ SO_4 + H_2SO_4$

can further increase the concentration of free sulfuric acid.

At a low pH, metals like aluminum, manganese, calcium and magnesium become soluble, thus increasing the hardness of acid mine water.

3.3 Acid Mine Water Pollution Control

The research on acid mine water typically follows two different paths: prevention of acidity formation, and elimination of acidity once it is formed.

3.3.1 Prevention of Acid Formation (7)

3.3.1.1 Air Sealing of Underground Mines

The purpose is to prevent oxygen from reaching the mine; thus, wet seals, that allow discharge of water and prevent inlet

of air, are in operation or are planned. However, a perfect seal is impossible because the air can filter through the pores of the ground; furthermore, the mine "breathes" with changes in atmospheric pressure and this contributes to interchange the air in the mine. Actually, after an initial decrease, the oxygen concentration increases to about atmospheric level.

3.3.1.2 Water Diversion

Since water is another reactant, reducing the amount of water that passes through the mine will reduce the quantity of acid mine drainage.

3.3.1.3 Surface Mine Reclamation and Revegetation

Surface mines are regraded and the most acidic material is buried. A layer of limestone can be added on top. Where possible, the land is revegetated; the purpose is to prevent erosion and to reduce the contact of oxygen and water with acidic materials.

3.3.1.4 Bacterial Activity Inhibition (8)

Chemical inhibitors or bacteria (Caulobacter) can be used to reduce the activity of Thiobacillus-Ferrobacillus and, therefore, to reduce the acidity production. This process is still in the laboratory stage and was never applied on a larger scale.

3.3.2 Pollution Abatement

3.3.2.1. Neutralization

Neutralization is the most used process; a neutralizing agent like lime, limestone or dolomite increases the pH of acid mine water (9).

Ferric and aluminum hydroxide and some $CaSO_4$ precipitate are separated in a settler.

Ferrous hydroxide can be oxidized with atmospheric oxygen and transformed into $Fe(OH)_3$.

The process is quite simple, but there are still some disadvantages involved:

- the water effluent from the plant contains up to 2000 ppm of $CaSO_4$, because $CaSO_4$ is not completely insoluble; this produces water of poor quality
- the neutralizing agent can be readily coated with precipitated salts, thus lowering the efficiency of the neutralization
- Figure 1 shows the acidity curve of Norton acid mine water; a great amount of neutralizer is required to precipitate iron and aluminum hydroxide at a pH about 3.0 and 4.4, respectively

- the precipitate is very soft and the slurry contains only 1% of solids. This can create problems in the handling and disposing of solids.

3.3.2.2 Other Processes

Other processes like reverse osmosis (10), multiflash distillation (3), crystallization (3), electrodialysis (3), ion exchange (11) are being studied either in laboratory or on pilot plant scale.

These processes are similar to those used for desalinization of sea water. Often they are coupled with a neutralization step.

The result is a pure water with a concentrated brine of salts and sulfuric acid as a by-product. The disadvantages are:

- a cost that is five to ten times higher than simple neutralization
- a serious corrosion problem
- the handling and the treatment of the resulting brine.

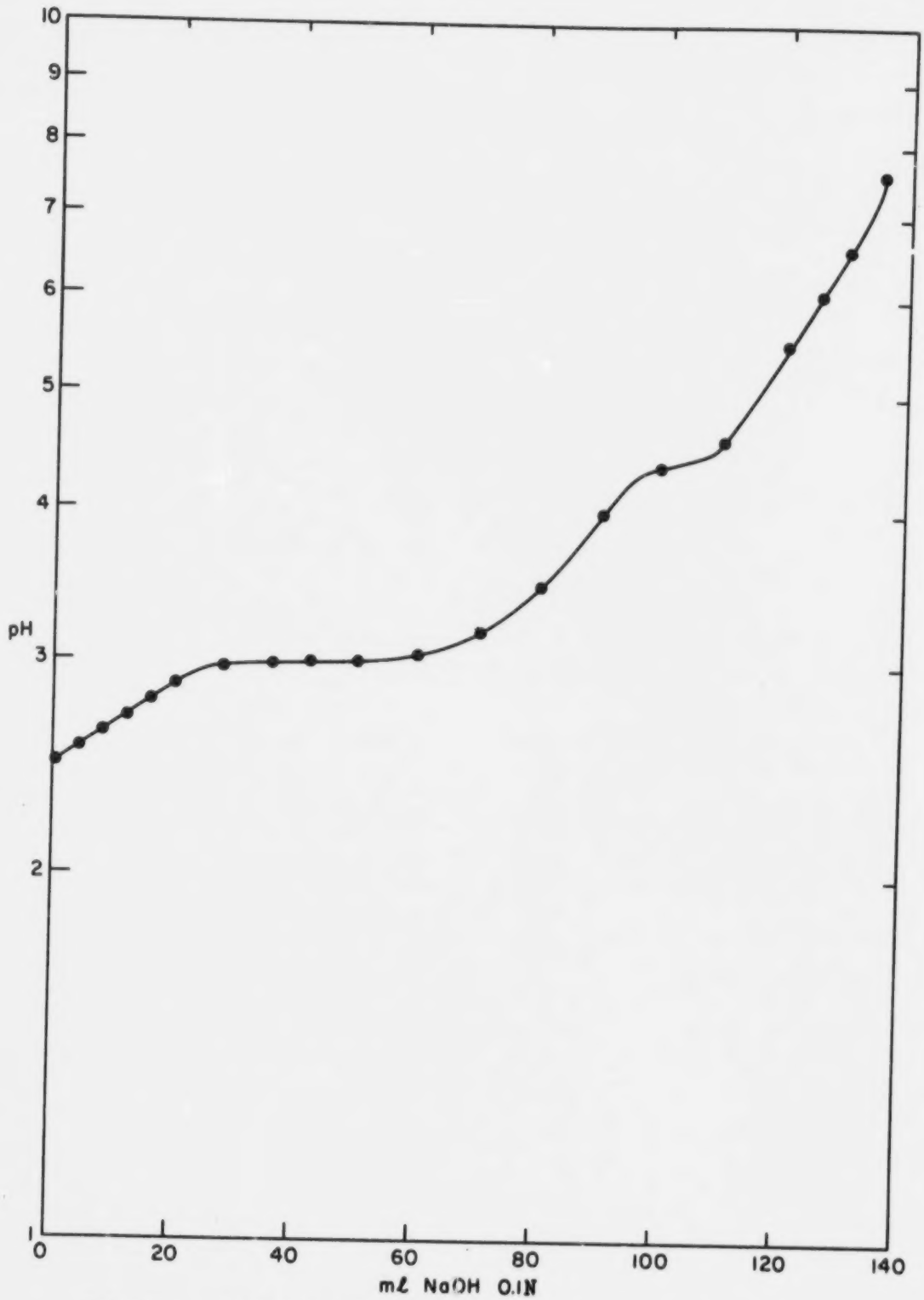


FIGURE 1 ACIDITY CURVE OF NORTON ACID MINE WATER

4. BIOCHEMICAL TREATMENT OF ACID MINE WATER

4.1 Introduction

Biochemical treatment of acid mine water is a possible alternative method for pollution abatement. Some anaerobic bacteria use sulfate ions as an oxidizing agent for their respiration; the end product of their respiration is hydrogen sulfide, which reacts with iron ions and forms insoluble ferrous and ferric sulfide, according to the following reactions:

$$Fe^{++} + SO_4^{--} + 8\ H^+ + 8\ e^- = FeS\downarrow + 4\ H_2O$$

$$2\ Fe^{+++} + 3\ SO_4^{--} + 24\ H^+ + 24\ e^- = Fe_2S_3\downarrow + 4\ H_2O$$

The second reaction is less likely to occur, because under the condition of the reaction, iron is present mainly in a reduced state. These reactions consume hydrogen ions; therefore, there is a reduction of acidity. For these reactions to take place, an electron source must be present. Organic salts can deliver electrons by decomposition.

4.2 Sulfur-Reducing Bacteria

Sulfate reducing bacteria are those bacteria, whose major energy-yielding metabolic reactions are linked to the reactions of the sulfate to the sulfide ions.

4.2.1 Classification

The classification of sulfate-reducing bacteria has been organized by Campbell and Postgate (12,13) and falls into two main groups:

(a) Genus Desulfomaculum

It is a spore-producing type and has three species: Dm., Orientis, Dm. Ruminis and Dm. Nigrificans.

(b) Genus Desulfovibrio

It is a non-sporulating, mesophilic type and has five species: D. Desulfuricans, D. Vulgaris, D. Africanus, D. Gigas and D. Salexigens. The strain D. Desulfuricans ATCC-7757 from American Type Culture Collection, was used throughout this study.

4.2.2 Ecology

D. Desulfuricans are very common bacteria: they are found either on land or in the sea (14).

Their natural habitat embraces a wide range of salinity, temperature, hydrostatic pressure, pH, redox potential. They are found in soil, sewage, fresh water, oil and sulfur deposits, drilling muds, industrial wastes and cooling water, in marine deposits and around buried iron structures.

4.2.2.1 Temperature Range of Growth

Sulfate reducing bacteria have been observed to grow over a wide range: on the lower range, some marine bacteria have been reported to grow slowly at -11°C. At the other extreme, some strains from deep oil and sulfur wells, reproduced at 104°C and 1000 atmospheres. However, D. Desulfuricans grow best around 25 to 45°C.

4.2.2.2 Pressure

Some strains can grow at atmospheric pressure and their activity stops completely at 400 atmospheres. However, some strains taken from deep wells grew only when compressed from 700 to 1000 atmospheres.

4.2.2.3 Optimum pH

pH tolerance of sulfate reducing bacteria can be affected by the chemical composition of the medium. However, they grow best at a pH around neutrality. Optimum growth was reported from pH 6.2 to 8. Tuttle, Dugan and Randles report activity at pH 2.8 with sawdust as a substrate. However, they think that most of the growth occurred in a thin layer around the sawdust grain where the pH is alkaline (15).

4.2.2.4 Redox Potential (16)

There are three major subtypes of metabolisms in which chemical-reduction reactions provide the ultimate source of energy.

(a) Aerobic respirations:	molecular oxygen is the ulti-electron acceptor
(b) Anaerobic respiration:	inorganic salts are the ultimate acceptors
(c) Fermentation:	organic compounds are the ultimate electron acceptors

Desulfovibrio Desulfuricans are strict anaerobes. They require a redox potential of about -200 mV for initiation of growth. After the growth is established, the bacteria produces enough H_2S to keep reducing conditions. Sulfate is the most common electron acceptor of D. Desulfuricans metabolism.

Other inorganic salts like sulfite, thiosulfate, tetrathionate and colloidal sulfur (but not elemental sulfur) can serve as electron acceptors and be reduced to sulfide (17). Reaction rates can vary greatly, not only with different environments and strains of bacteria, but also with the age and physiological conditions of the bacteria.

4.2.2.5 Carbon Source

D. Desulfuricans can use a large variety of organic carbon compounds such as lactate, malate, citrate, pyruvate, tartrate, fatty acids, alcohols and some aminoacids as a source of energy and electrons.

There are some reports about a possible autotrophic activity; that is, D. Desulfuricans could be able to metabolize inorganic compounds like CO_2, H_2 and N_2 (18). In our study sodium lactate was the principal organic source of carbon.

According to Postgate and Grossman, the reaction of lactate follows this path (19):

$$(CH_3)(HCOH)(COOH) \xrightarrow{1/4\ H_2SO_4} (CH_3(CO)(COOH) + H_2O$$

lactate — pyruvate

$$(CH_3)(CO)(COOH) \xrightarrow{1/4\ H_2SO_4} (CH_3)(COOH) + CO_2$$

pyruvate — acetate

Lactate is oxidized quantitatively to acetate and CO_2, while SO_4^{--} is reduced to S^{--}.

4.2.2.6 Nitrogen Supply

Nitrogen is supplied by ammonium salts and amino acids. Other nitrogen compounds like nitrate salts are not metabolized by D. Desulfuricans.

4.3 Bacterial Kinetics

Bacterial kinetics is affected by many variables, such as environmental conditions, concentration of nutrients, age of bacteria, and so on. In order to have simple mathematical equations, bacterial kinetics are described by partial models in which a limited number of significant variables is considered.

4.3.1 Michaelis-Menten Equation

One of the most widely accepted models of enzyme kinetics is that of Michaelis-Menten (20). These workers represented the reaction of enzyme with substrate as follows:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E - S$$

$$E - S \xrightarrow{k_{+2}} E + P$$

where

E = enzyme

S = substrate

E-S = enzyme-substrate complex

P = product

k_{+1} = forward reaction constant

k_{-1} = reverse reaction constant

k_{+2} = reaction rate constant

Denoting e, S, and C as the concentration of total enzyme, substrate, and enzyme-substrate complex, the rate of change will be

4.3.1.1. $$\frac{dC}{dt} = k_{+1} (e-C) S - k_{-1} C - k_{+2}$$

If we assume that the value of S is much greater than that of E, then at steady-state

4.3.1.2 $$C = \frac{eS}{\frac{k_{-1} + k_{+2} + S}{k_{+1}}} = \frac{eS}{K_m + S}$$

The rate of product formation is given by:

4.3.1.3 $$r = \frac{dP}{dt} = k_{+2} C = \frac{k_{+2} eS}{K_m + S} = \frac{QS}{K_m + S}$$

where

Q = maximum rate when all the enzyme forms the enzyme-substrate complex

K_m = value of S, when the reaction proceeds at half of the maximum reaction rate

4.3.2 Monod Equation

In the case of unicellular growth, the rate of growth can be expressed by a limiting substrate concentration as follows:

4.3.2.1 $$\frac{dX}{dt} = f(X,S)$$

where

X = mass of cells per unit volume

Let,

$$\mu = \frac{1}{X} \frac{dX}{dt}$$

the specific rate of growth, then, according to Monod, the following relationships exist:

4.3.2.2 $$\mu = \bar{\mu} \left(\frac{S}{K_s + S}\right)$$

$\bar{\mu}$ = maximum rate of growth when S is unlimited

K_s = substrate concentration at which the specific growth rate is one-half the maximum value

4.3.3 Yield

There are different definitions of yield:

Yield of Growth

$$Y_{x/s} = - \Delta X/\Delta S$$

(Increase in biomass concentration for a unit decrease in substrate concentration)

Yield of Product Based on Cell Mass

$$Y_{p/x} = \Delta P/\Delta X$$

(Increase in product concentration for a unit increase in biomass concentration)

Yield of Product Based on Limiting Substrate

$$Y_{p/s} = - \Delta \Gamma/\Delta S$$

(Increase in product concentration for a unit decrease in substrate concentration)

4.3.4 Continuous Fermentation

Continuous fermentation was initiated about 50 years ago when fodder yeast was produced by a continuous process. Since then, continuous cultures of micro-organisms have been carried out by a great number of workers. Continuous cultures have been favored for physiological studies primarily because environmental conditions can be kept constant, in sharp contrast with batch cultures, where micro-organisms are subject to continual change.

Although many studies of yeast, bacteria and fungi in continuous fermentation in laboratories have been published, industrial scale applications are scarce. The principal difficulties of operating continuously are: the possibility that the desired microbial species might undergo deleterious or unwanted mutations and the technical difficulty of running aseptically for a long period of time.

Consider a single-stage perfectly mixed culture system. Let the continuous culture contain a constant volume V, with cell concentration X and substrate concentration S. Fresh culture medium of substrate concentration S_o is fed at a steady flow rate F.

Then, we can write the following material balance at steady-state.

4.3.4.1 Biomass:

In + Generated = Out (In = 0 in our case)

4.3.4.2 $$V \frac{dX}{dt} = V\mu X = FX$$

4.3.4.3 If Monod equation is applied:

$$V\bar{\mu}X \frac{S}{K_s + S} = FX$$

By dividing both terms by VX and defining D = F/V (dilution ratio):

4.3.4.4 $$D = \bar{\mu} \frac{S}{K_s + S}$$

For the substrate, the following material balance can be written:

4.3.4.5 In - Consumed = Out

or

4.3.4.6 $$F S_o - V\left(\frac{dS}{V\,dt}\right) = F \cdot S \ ,$$

but using the yield concept, it is possible to write

$$\frac{1}{V}\frac{dS}{dt} = -\frac{1}{Y_{x/s}}\frac{dX}{dt} = -\frac{\mu X}{Y_{x/s}}$$

and substituting into equation 2.3.4.6.

4.3.4.7 $$F S_o - \frac{V}{Y_{x/s}} \mu X = FS$$

If Monod equation is applied, equation 4.3.4.7 can be arranged to give

4.3.4.8 $$D(S_o - S) = \frac{\bar{\mu}\, X \cdot S}{Y_{x/s}\,(K_s + S)}$$

The equations 4.3.4.4 and 4.3.4.8 describe the behavior of a continuous culture.

4.3.5 Kinetic Analysis

The purpose of a kinetic analysis is to determine the growth constants $\bar{\mu}$, K_s and $Y_{x/s}$. $Y_{x/s}$ can be determined directly, but for $\bar{\mu}$, and K_s, it is useful to use the Lineweaver-Burk plot. If we take the reciprocals of equation 4.3.4.4,

4.3.5.1 $$\frac{1}{D} = \frac{K_s}{\bar{\mu}} \cdot \frac{1}{S} + \frac{1}{\bar{\mu}}$$

then $\bar{\mu}$ and K_s can be determined by plotting 1/D vs 1/S and measuring the absolute value of the reciprocals of the intercepts on the two axis.

If $K_s >> S$, equation 2.3.4.4 becomes

4.3.5.2 $$D(S_o - S) = \frac{\bar{\mu}}{K_s Y_{x/s}} XS$$

and substituting equation 4.3.5.2 into it, equation 4.3.5.3 reduces to

4.3.5.3 $$(S_o - S) = X/Y_{x/s} \ ;$$

that is, the definition of yield, when there is no biomass fed into the reactor.

Another case is the case in which there is a consistent culture on the wall of the reactor. In this case, equation 4.3.4.5 becomes for the substrate,

4.3.5.4 In - (consumed in the reactor) = out ,

But in this case, the substrate can be consumed in the reactor and on the wall.

4.3.5.5 $$F S_o - V\left(\frac{dS_r}{V\,dt}\right) - W\left(\frac{dS_w}{W\,dt}\right) = FS$$

where the subscript r stands for reactor and w for wall, respectively. If the amount of substrate consumed on the wall is much greater than the amount consumed in the reactor, the equation becomes independent from the concentration of bacteria in the reactor (X) and can be written as

4.3.5.6 $$F(S_o - S) = dS_w/dt$$

dS_w/dt can be expressed as a Michaelis-Menten equation type and equation 4.3.5.6 becomes

4.3.5.7 $$F(S_o - S) = \frac{\bar{Q}_w S}{K_w + S} \quad \text{where } Q_w = \frac{\bar{Q}_w}{V}$$

This equation has the same shape of equation 4.3.4.7 in which X is constant and

$$Q_w = \frac{\bar{\mu} X}{Y_{x/s}} .$$

This equation can be analyzed with the Lineweaver-Burk plot to determine the constants. In this case, we plot $1/D\ (S_o - S)$ vs $1/S$ and Q_w and K_w are determined as the absolute values of the reciprocals of intercepts on both axis.

It is interesting to note that in this case the rate of reduction of substrate concentration does not depend upon bacterial concentration in the culture; therefore, there could be a reaction even when all the bacteria of the culture have been washed out. Naturally, this effect should be more conspicuous at high ratios surface/volume and at high dilution ratios, when the effect of the volume becomes small, because bacteria in solution are washed out.

If bacteria are not washed out and their number in solution is much higher than on the wall, equations 4.3.4.4 or 4.3.5.2 govern the reactor independently of equation 4.3.5.7.

5. EXPERIMENTAL PROCEDURES

5.1 Stock Culture

D. Desulfuricans ATCC-7757 from the American Type Culture Collection was used throughout this work. Stock cultures were maintained on a lactate medium in screw-capped tubes and kept at a temperature of about 4°C.

The composition of the lactate medium is listed in Appendix 1. Periodically, the culture was transferred to a fresh medium. Also, solid media are used for stock cultures in screw-capped tubes at 4°C.

For routine subcultures, the lactate medium was put into 25 x 200 mm screw-capped tubes and sterilized by autoclaving for fifteen to twenty minutes at 15 psig. Then 1 ml of stock culture was added to each tube. These tubes were incubated at 37°C ± 1 C until full growth developed and then were stored at 4°C. When the experiments were made on solutions with low sulfate initial concentration, the stock culture and routine subcultures (with the Postgate medium C and half concentration of salts) were prepared, as shown in Appendix II.

A fresh sterilized medium was added to stock cultures to restore the original volume.

The pH of fresh culture was adjusted to the desired value with 0.1 N HCl or with 0.1 N NaOH.

5.2 Analytical Determinations

Sulfate was measured turbidimetrically as $BaSO_4$ precipitate as described in Appendix VIII, with a Coleman Junior II Spectrophotometer at 420μ. Oxidation-reduction potential was determined with a Beckman Zeromatic SS-3 pH meter.

pH was measured with a Beckman Zeromatic pH meter. The cell concentration was measured as an optical density with the Coleman Junior Spectrophotometer at 525μ with distilled water as a blank.

Dry weight measurements were made by passing a volume of 100 ml of culture through a previously weighed Seitz Filter with an average pore size of 0.01μ using a Seitz filtration apparatus.

The filter and collected cells were then stored at 80°C, and weighed every 24 hours until a constant weight was obtained. The dry weight of organisms was calculated as the weight increase of the filter.

Total Carbon, Inorganic Carbon and Organic Carbon concentration was measured with a Beckman Model 915 Total Organic Carbon Analyzer.

6. DESCRIPTION OF EXPERIMENTS AND RESULTS OF BATCH RUNS

6.1 Batch Runs with Artificial Media

The original Postgate medium was modified: iron sulfate for the whole series of experiments was kept at 2-5 ppm, instead of 0.1 g/l in order to avoid the blackening of the solution that could interfere with sulfate determination. Then the Postgate medium was diluted and modified in a series of batch runs in order to come closer to the average sulfate concentration in acid mine water. Na_2S was added to lower the oxidation-reduction potential and thus to reduce the time lag before growth.

6.1.1 Runs at Full Concentration

A set of runs was made with different initial concentrations of sodium lactate. The initial concentration of sodium lactate was, respectively, no lactate, 250 ppm, 500 ppm, 1000 ppm, 3000 ppm. The composition is shown in Appendix III. In this set of runs yeast extract concentration was lowered from 1.0 g/l to 0.5 g/l. The different media were put in screw-capped tubes, sterilized for fifteen minutes at 15 psig, inoculated with 1 ml of stock culture and incubated at 37°C and initial pH 7. The results are shown in Figure 2.

Sulfate reduction is proportional to initial lactate concentration. In addition, the final inorganic carbon concentration is proportional to the initial organic carbon concentration. The slope of the line is 0.31 (moles inorganic carbon)/(moles initial organic carbon) which is close to the theoretical value of 0.33, given by the lactate reaction alone.

Sodium citrate and yeast extract evidently take part in the reaction and constitute part of the organic substrate as well as lactate.

Sulfate reduction is proportional to the final inorganic carbon concentration.

The slope of the line is 0.47 (moles sulfate reduced)/(moles inorganic carbon) which is close to the theoretical value of 0.5 (moles sulfate reduced)/(moles inorganic carbon produced).

6.1.2 Runs with Diluted Postgate Medium

The original Postgate medium C was diluted at half and a quarter concentration, sterilized, inoculated with 1 ml of stock culture and incubated at 37°C, with initial pH 7 in screw-capped tubes. The results are shown in Table 3.

In both cases, sulfate reduction was almost quantitative.

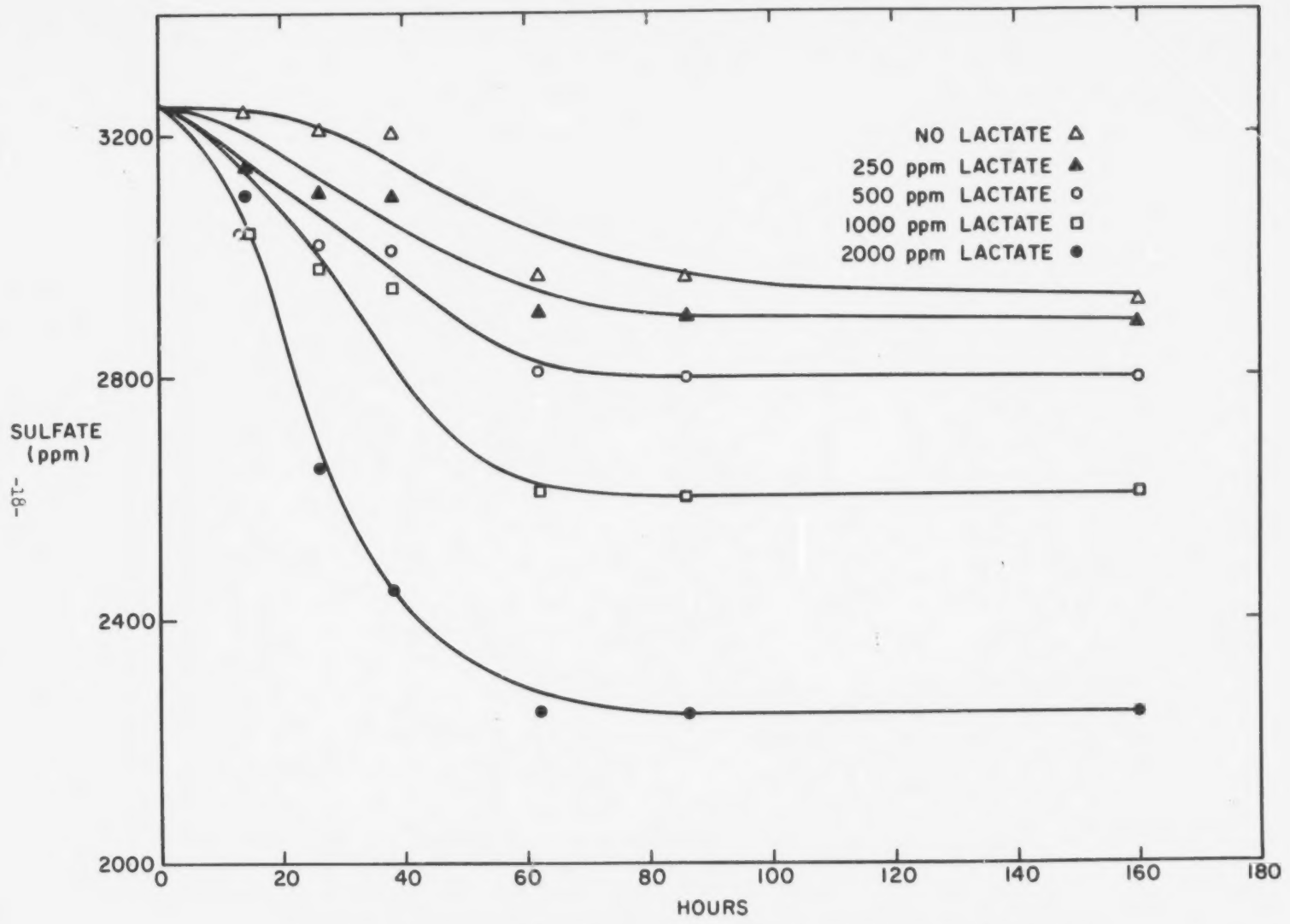
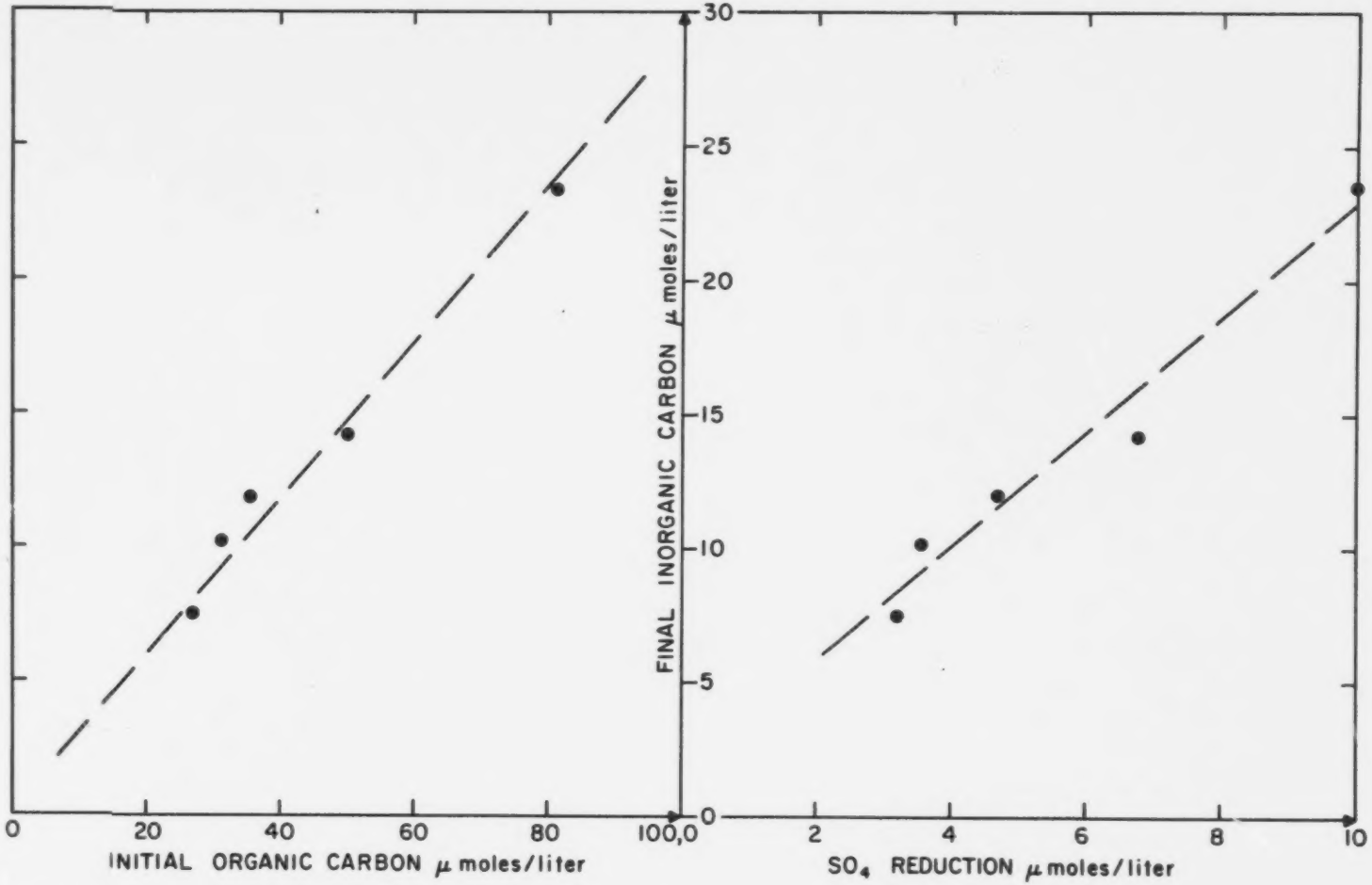


FIGURE 2 SULFATE REDUCTION AT DIFFERENT LACTATE CONCENTRATION



INITIAL ORGANIC CARBON vs FINAL INORGANIC CARBON FINAL INORGANIC CARBON vs SULFATE REDUCTION

FIGURE 3

Table 3 - Runs with Diluted Postgate Medium

Time (hours)	Sulfate (ppm)	
0	1600	800
14	1700	820
43	715	60
67	60	60

This experiment demonstrates that the reaction can go almost to completion, if enough organic substrate is present. From that point on, all experimental work was conducted with the Postgate medium C diluted at half concentration.

The initial concentration of sulfate is about 1600 ppm, that is, similar to an average concentration of acid mine water. The composition of this medium is listed in Appendix II.

6.1.3 Yeast Extract and Temperature Effect

A set of runs was made with different initial yeast extract concentration at 20°C and 37°C.

The sterilized medium was inoculated with 0.5 ml of stock culture and incubated in screw-capped tubes with initial pH 7. The initial composition is listed in Appendix III. The results are shown in Figure 4.

Apparently, yeast extract did not have a significant effect on the rate of reaction. However, in all cases, solutions with lower yeast extract concentrations had a longer time lag, but after the reaction started, the rate was independent of the yeast extract concentration.

The biggest influence is due to temperature. At 20°C the time lag was approximately four times the time lag at 37°C.

The sulfate reduction rate at 37°C was about 60 ppm SO_4^{--}/hour, while at 20°C it was about 30 ppm SO_4^{--}/hour, which is only one-half of the reduction rate at 37°C.

6.1.4 Lactate Concentration Effect

A set of runs was made with different initial lactate concentrations at 37°C and initial pH 7*. The sterilized medium was inoculated with 1 ml of stock culture and incubated in screw-capped tubes. The results are shown in Figure 5.

Sulfate reduction is proportional to the initial lactate concentration and can be almost quantitative if enough lactate is present. As in case 6.1.1, there is a slight reduction without lactate: yeast extract and citrate can replace lactate as organic substrates. Figure 6 shows the relationship between sulfate reduction and initial lactate concentration as it resulted from runs 6.1.1 and 6.1.4.

*The initial composition is listed in Appendix III.

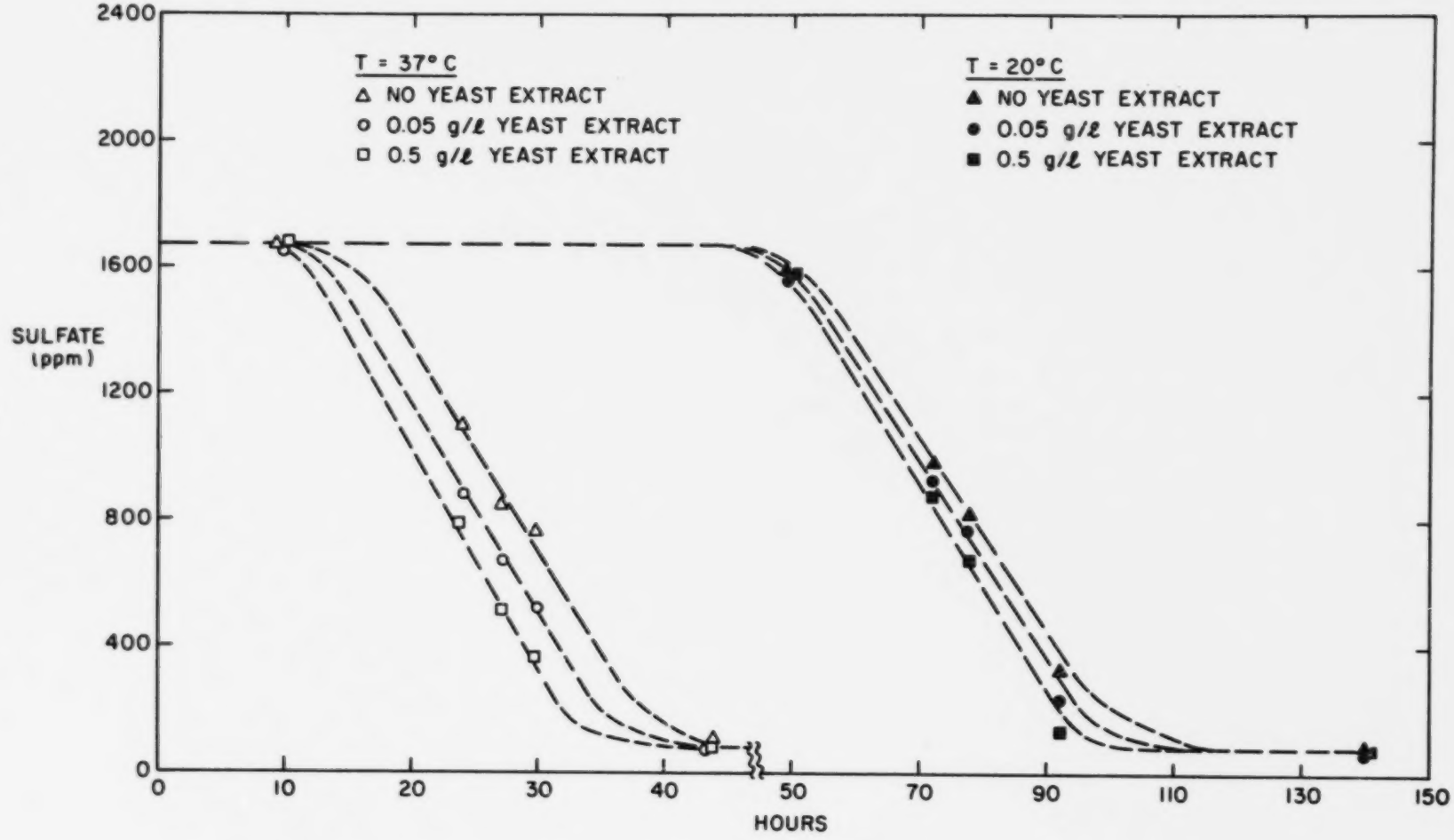


FIGURE 4 SULFATE REDUCTION AT DIFFERENT TEMPERATURE AND YEAST EXTRACT CONCENTRATION

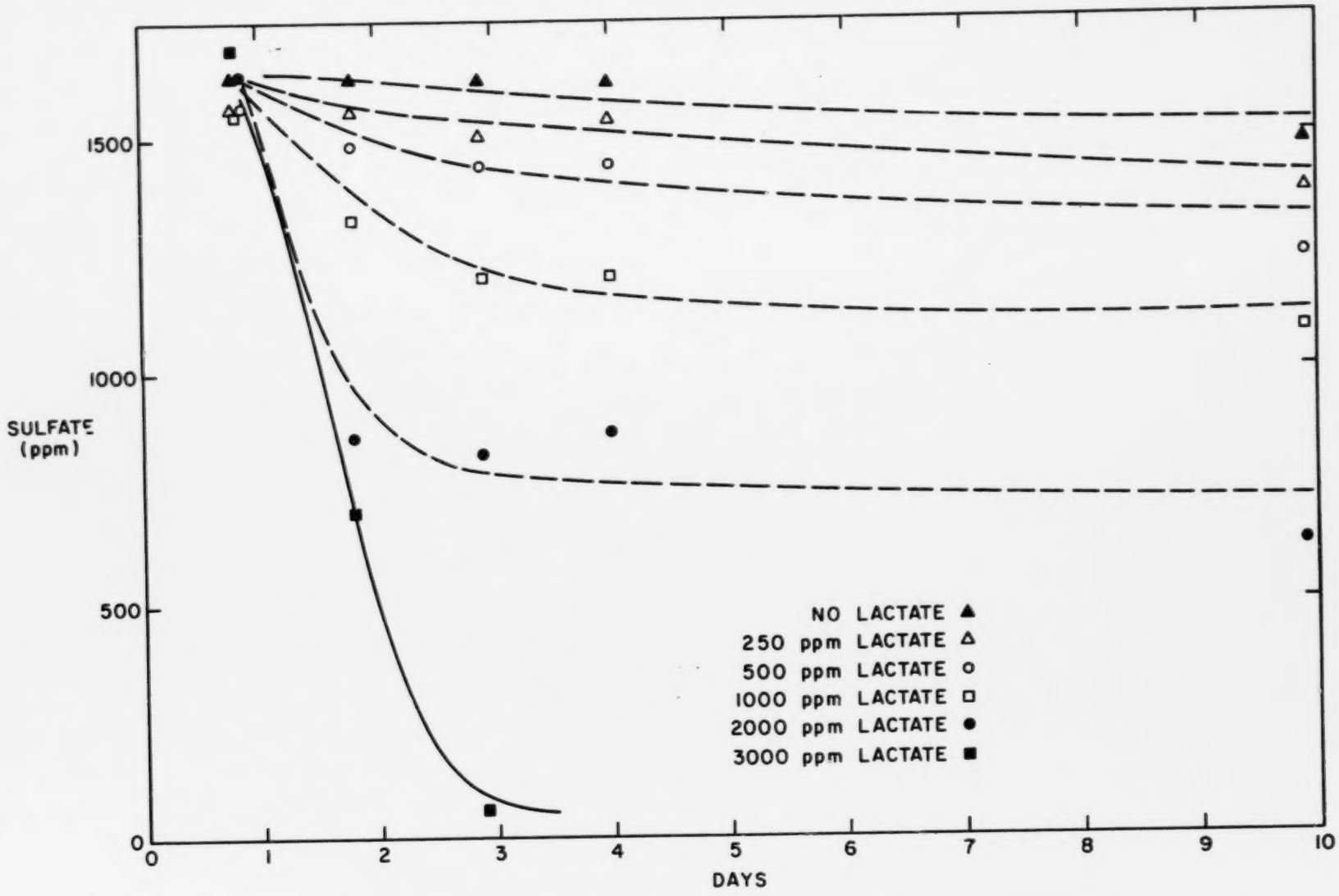


FIGURE 5 SULFATE REDUCTION AT DIFFERENT LACTATE CONCENTRATION

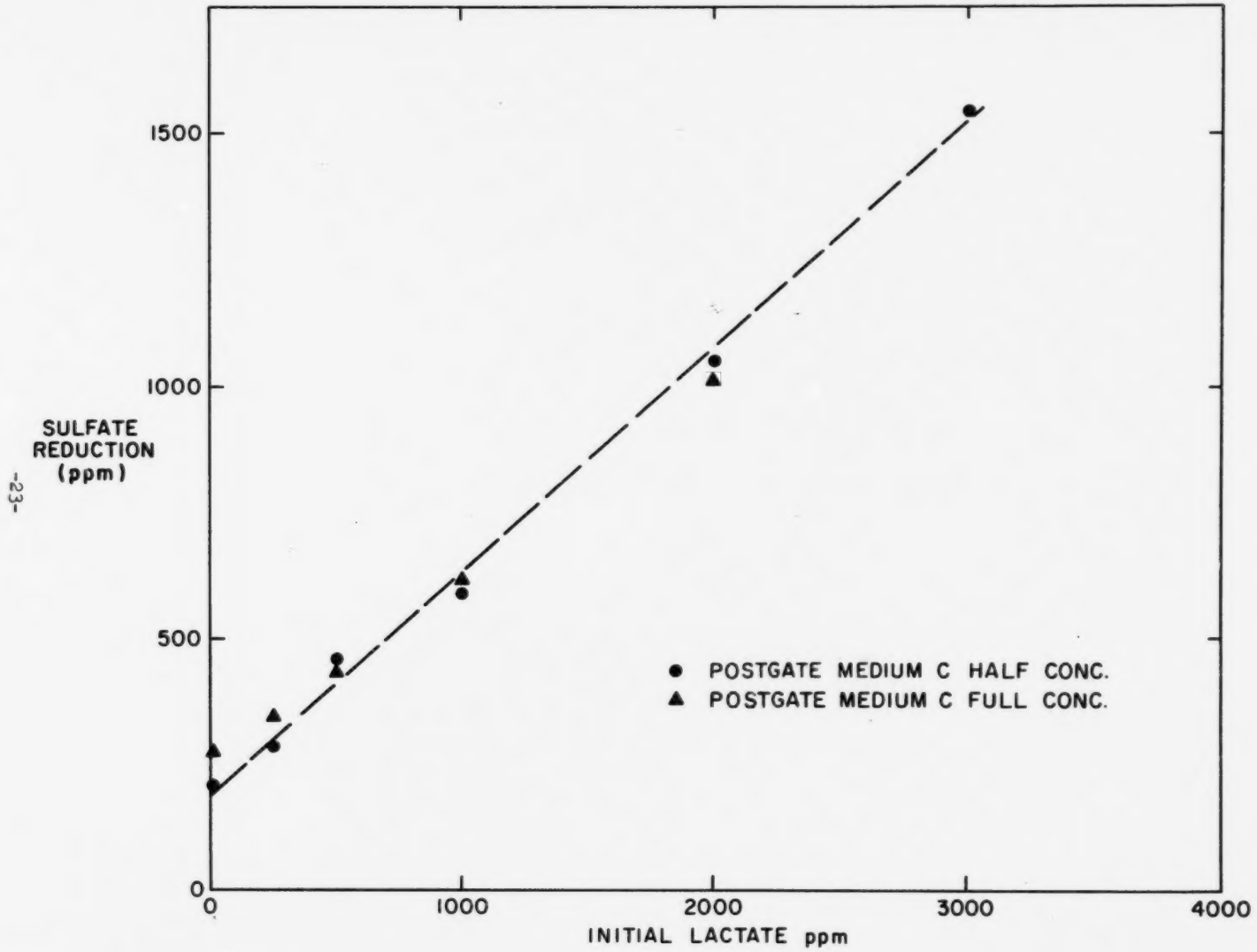


FIGURE 6 SULFATE REDUCTION vs INITIAL LACTATE CONCENTRATION

6.2 Batch Runs with Digester Sludge

Sodium lactate is an expensive chemical. Its cost ranges from 14¢/lb. for 50% technical grade to 52¢/lb. for the 60% edible grade.

Therefore, other organic substrates like: digester sludge, raw sludge from sewage treatment plants, milk whey or milk (to simulate dairy wastes), "Trastan" (to simulate pulp and paper industry wastes), sugar (to simulate molasses) were tested as possible organic substrates. For the digester sludge runs, salts were dissolved in the sludge, and the resulting solution had the composition listed in Appendix IV.

The solution was adjusted to the desired pH and redox potential. No sterilization took place in any run with digester sludge. The carbon content of the solution was always a little lower than the expected value; evidently, some organic compounds precipitate upon mixing digester sludge with salts.

6.2.1 Effect of Temperature and Initial pH

A first set of runs was made at 15.5°C and 37°C with different initial pH. The solution was inoculated with 1 ml of stock culture and incubated in screw-capped tubes. The results are shown in Figure 7.

Sulfate reduction increases with the increase of temperature and initial pH. Temperature appears to be the dominant variable.

6.2.2 Effect of Organic Carbon Concentration

The digester sludge medium was diluted twice and then four times, inoculated with stock culture and incubated at 37°C in screw-capped tubes.

The initial composition is listed in Appendix V. The results are shown in Table 4.

Table 4 - Data of Run 6.2.2

Time (Hours)	Sulfate (ppm)		
0	800	1600	3150
24	880	1600	2850
72	820	1620	2840
108	700	1280	2600
300	700	1320	2620

Sulfate reduction is proportional to the amount of carbon initially present and the slope of this curve is higher than the slope of the run with lactate. This means that apparently organic carbon from sludge is a more efficient substrate for a culture of D. Desulfuricans than lactate. However, no definite conclusion can be drawn, because the carbon concentration is measured in the liquid phase, and, therefore, it does not take into consideration the solid phase suspended in the digester sludge that can be degraded and metabolized by

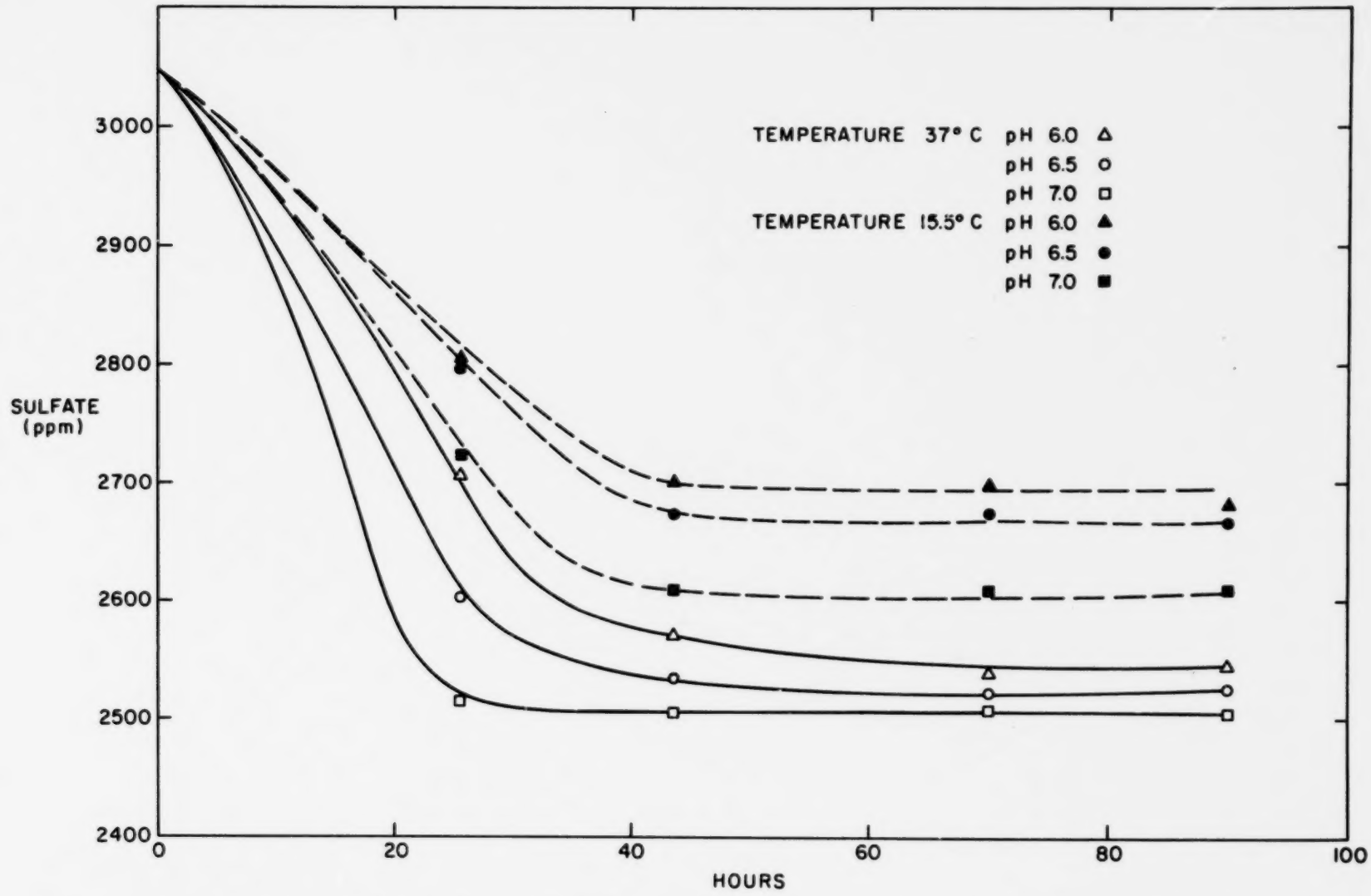


FIGURE 7 SULFATE REDUCTION AT DIFFERENT TEMPERATURE AND INITIAL PH (DIGESTER SLUDGE MEDIUM)

D. Desulfuricans. Moreover, yeast extract and sodium citrate might have contributed to the reaction to an unpredictable extent. Table 5 shows the change in carbon concentration and pH.

Table 5 - Carbon Concentration and pH in Run 6.2.2

Tube		Total Carbon (ppm)	Inorganic Carbon (ppm)	Organic Carbon (ppm)	pH
1	initial	167	100	67	7.0
	final	101	91	10	7.4
2	initial	265	166	100	7.0
	final	228	156	72	7.4
3	initial	485	265	191	7.0
	final	330	295	35	8.0

Another experiment was made in a 1 liter-flask with a solution having the composition listed in Appendix VI. Operating procedures were the same as in 6.2.2. Table 6 shows the results. There is a lag time of at least two weeks. The reaction seems to start with some difficulty and proceeds slowly only to a limited conversion.

Table 6 - Run in 1 Liter-Flask

Time (Weeks)	Sulfate (ppm)	Total Carbon (ppm)	Organic Carbon (ppm)	Inorganic Carbon (ppm)
0	1220	682	366	316
1	1165			
2	1220			
4	1070			
6	860	410	98	312
8	900			
16	800			

6.3 Experiments with Milk Whey

Milk whey was tested as a possible organic substrate. Milk was acidified with HCl in order to separate casein by filtration; then pH was raised to 7 by neutralization with NaOH.

Redox potential was lowered by adding Na_2S. Five solutions, whose composition is shown in Appendix VI, were prepared with different initial organic carbon concentrations. The solutions were sterilized, inoculated with 1 ml of stock culture and incubated at 37°C in screw-capped tubes. A screw-cap tube with Postgate medium was used as a control. No reduction was observed after ten days, while the reaction proceeded regularly in the control tube.

The experiment was repeated using natural milk whey. No significant reduction was observed after 28 days. A third experiment was performed using natural milk whey, that was previously filtered by Seitz filtration to avoid any possible interference in sulfate determination, but even in this case no reduction was observed after 14 days.

6.4 Run with Trastan

A solution was prepared by dissolving Trastan with some salts. Trastan is a dried waste sulfite liquid containing lignite as the primary organic carbon source. The composition is listed in Appendix VII.

pH was 7.15 and redox potential was adjusted with Na_2S. The solution was inoculated with 1 ml of stock culture and incubated at 37°C in a screw-capped tube. No reduction was observed after one month.

6.5 Run with Sugar

A solution was prepared by dissolving sugar in a solution of salts. The composition is listed in Appendix VII. pH was adjusted to 7 and redox potential was reduced with Na_2S. The solution was inoculated with 1 ml of stock culture and incubated at 37°C in screw-capped tubes. No reduction was observed after two weeks, while a control tube with the Postgate Medium C reacted normally.

7. EXPERIMENTS WITH ACID MINE WATER

7.1 Introduction

In the previous chapter experiments were made with artificial solutions, with different organic substrates. In this chapter natural acid mine water is used together with different organic substrates.

Sewage sludge is used extensively as organic substrate either alone or with other organic compounds. Sewage sludge was tested first among other possible natural organic substrates, because of some encouraging results in the previous chapter and also because it is a cheap source of organic carbon. The composition of different acid mine waters was listed in Table 1. During these experiments, no medium was sterilized.

7.2 Mixing of Digester Sludge with Acid Mine Water

Upon mixing digester sludge with acid mine water there is an increase in pH and a reduction in oxidation potential.

7.2.1 Proctor 1 Acid Mine Water and Digester Sludge

The initial and the final conditions are summarized in Table 7.

Table 7 - Data of Experiment 7.2.1

	SO_4^{--} (ppm)	pH	redox pot.(mV)
Proctor 1 AMW	250	3.1	105
Digester Sludge	negligible	7.75	-155
1 Sludge/4 AMW	120	6.88	- 45
1 Sludge/1 AMW	90	7.4	- 85
4 Sludge/1 AMW	60	7.65	-130

In all cases, upon mixing, there is a precipitate, probably of Al and Fe hydroxides. The three solutions were put in screw-capped tubes, inoculated with stock culture of Desulfovibrio and incubated for a week at 20 and 37°C. There was no significant reduction of sulfate. Only the solutions at 37°C showed a slight reduction in oxidation potential.

7.2.2 Bennett Acid Mine Water and Digester Sludge

The initial and the final conditions are summarized in Table 8.

Table 8 - Data of Experiment 7.2.2

	SO_4^{--} (ppm)	pH	Oxidation Potential (mV)
Bennett Acid Mine Water	290	3.0	380
Digester Sludge	negligible	7.75	-155
1 Bennett/1 D. Sludge	170	7.12	- 60
4 Bennett/1 D. Sludge	240	6.55	- 90

The two solutions were put in screw-capped tubes, inoculated and incubated for a week at 37°C. There was no meaningful reduction of SO_4^{--}. However, there was a slight increase in pH and a decrease in oxidation potential.

Figure 8 shows the acidity curve of Bennett Acid Mine Water, when neutralized with NaOH 0.1 N and also the pH curve obtained by mixing Bennet Acid Mine Water with a different sample of digester sludge.

7.2.3 Norton Acid Mine Water and Digester Sludge

Figure 9 shows the pH curve obtained by mixing Norton Acid Mine Water with Digester Sludge and Raw Sludge.

The increase in pH is greater with Digester Sludge. Also, at pH 3.1 there is the characteristic inflection point due to iron hydroxide precipitation. At ratio 4:1 the pH curve becomes almost asymptotic and never reaches the initial pH of digester sludge. However, at a 4:1 ratio the mixture has a pH high enough to start a viable culture of D. Desulfuricans. This does not happen with Raw Sludge: in this case, the pH must be raised to a convenient value by neutralization with some basic compound. Norton Acid Mine Water, however, has a very high acidity and a sulfate concentration of about 9555 ppm, which is about five times greater than the average acid mine water.

7.3 Norton Acid Mine Water and Digester Sludge

7.3.1 Digester Sludge Medium

A sample of Norton acid mine water was mixed with four parts of digester sludge and sodium sulfide was added to raise the pH from 6 to about 7 and to lower the reduction potential.

The mixture was then divided into three samples and each sample was put in a rubber stoppered 250 ml flask. In one flask 1 gram of sodium lactate was added. In the second flask 0.5 grams of yeast extract were added, while no chemical was added in the third flask. The flasks were inoculated with stock cultures of D. Desulfuricans and incubated at 37°C. The results are summarized in Table 9.

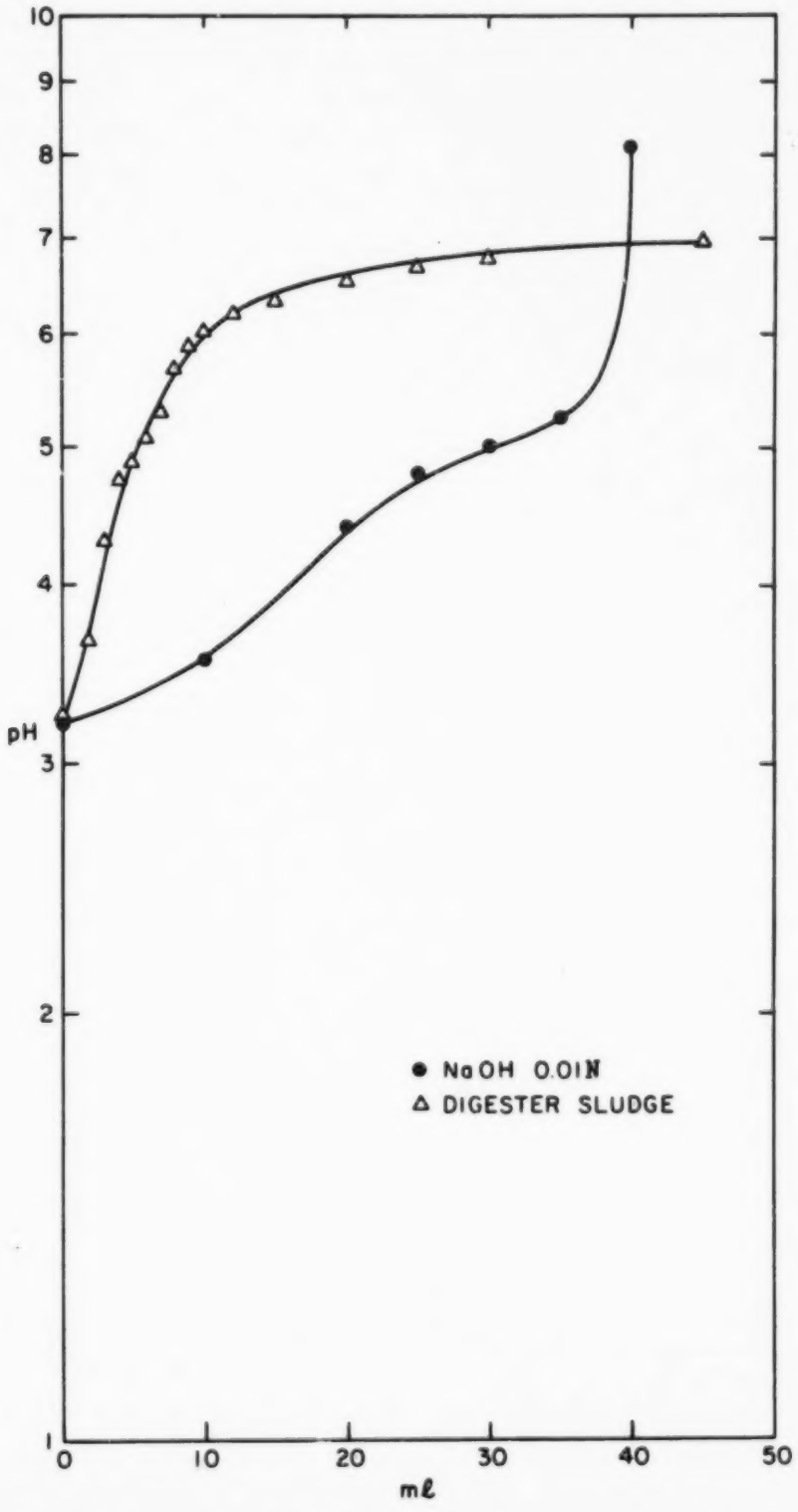


FIGURE 8 ACIDITY CURVE OF BENNETT ACID MINE WATER (100 mℓ)

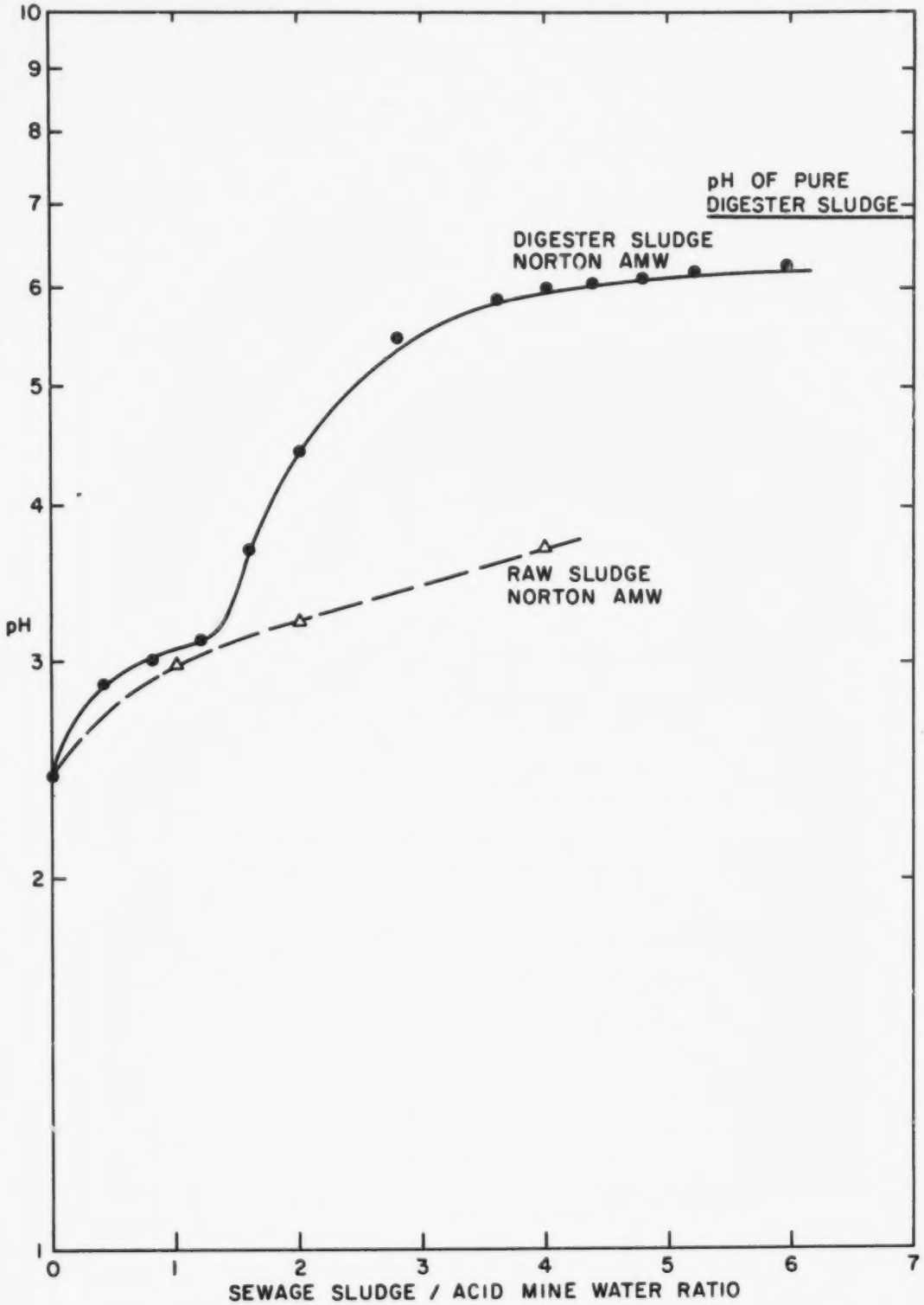


FIGURE 9 ACIDITY CURVE OF NORTON ACID MINE WATER

Table 9 - Data of Run 7.3.1

Time (days)	0	2	3	4	8*	15
1 (lactate medium)						
sulfate (ppm)	1390	1330	1270	320	330	320
redox (mV)	-160	-180	-360	-390	-365	-363
2 (yeast extract)						
sulfate (ppm)	1390	1400	1390	1410	1340	1270
redox (mV)	-160	-270	-360	-350	-355	-375
3 (no chemical added)						
sulfate (ppm)	1390	1390	1390	1380	1420	1440
redox (mV)	-160	-210	-250	-275	-170	-370

*At this point solutions two and three were inoculated again with stock culture.

Table 10 - Data of Run 7.3.2

Time (days)	0	1	3	4	5	6	28
2 (lactate added)							
sulfate (ppm)	1460	1460	1460	700	330	330	
redox (mV)	-140	-140	n.d	-370	-380	n.d	
3							
sulfate (ppm)	1460	1460	n.d	1390	1400	1330	1360
redox (mV)	-140	-140	n.d	-260	-260	n.d	

7.3.3.1 Acid Mine Water and Digester Sludge Alone

Norton acid mine water and digester sludge were mixed and put in a rubber stoppered 250 ml flask. The mixture was inoculated with a culture sampled from the run with the artificial medium described in 7.2.3. and incubated at 37°C. The results are summarized in Table 11.

Table 11 - Data of Run 7.3.3

Time (Days)	SO_4^{--} (ppm)	pH	Oxidation Potential (mV)
0	1490	5.85	- 60
10	1220	6.65	-220
21	120	7.15	-370

The run was repeated using the same mixture and a second mixture was prepared by adding filtered digester sludge to the acid mine water. The two mixtures were put into rubber stoppered 250 ml flasks and inoculated with a culture taken from the run described in 7.2.3 and incubated at 37°C.

In this case, a slight reduction (from 1240 ppm SO_4^{--} to 1100 ppm SO_4^{--}) was observed in about four weeks in the first mixture. No reduction was observed in the mixture made with filtered digester sludge.

A possible interpretation of these contradictory results is that the reduction is affected more by the quantity of organic solids suspended in the sludge than by the organic carbon dissolved in the sludge (303 ppm); that is, samples of sludge, apparently homogeneous in dissolved organic carbon, can behave in a different way because they are not homogeneous with respect to suspended organic solids.

There is a substantial reduction in lactate medium, while the reaction proceeded slowly with yeast extract medium and there was no reduction at all with no chemical added. The reduction in oxidation potential is not always connected with a sulfate reduction. This can be due to other anaerobic bacteria, such as methane bacteria, that can find suitable nutrients and viable conditions in a non-sterilized culture.

7.3.2 Raw Sludge Medium

A second experiment was performed with Norton acid mine water and raw sludge, sampled after the grit removal section of the sewage treatment plant in Syracuse. As shown in Figure 9, upon mixing acid mine water and raw sludge there is no substantial increase in pH and also the oxidation potential is not affected and oxidating (aseptic) conditions prevail.

Thus, it is necessary to add Na_2S, in order to increase the pH and lower the oxidation potential to a convenient value. The mixture was divided into two parts and put into rubber stoppered 250 ml flasks. To the first flask, 1 gram of sodium lactate was added, while none was added to the second. Only the flask with lactate added showed significant sulfate reduction.

8. SEMIBATCH RUN

8.1 Introduction

Batch runs in media obtained by mixing acid mine water with digester sludge, gave contradictory results. Some authors (21), however, have pointed out that there could be a process of adaptation to sludge by D. Desulfuricans. Therefore, experiments were performed in which a culture was started with an artificial medium (Postgate medium) and then, by changing one component at a time, a viable culture was obtained in a medium consisting of a mixture of acid mine water and digester sludge alone.

8.2 Experimental Procedure

A semibatch run was chosen, because it was simple to remove and to add small quantities of slurry. Two runs were made in parallel: one at 37°C in an incubator and one at room temperature (about 20°C). The reactors were two glass stoppered flasks of 200 ml. In the first period (18 days) every 12 hours, the flasks were shaken, in order to have a homogeneous suspension, then 50 ml of solution were withdrawn and substituted with an equal amount of fresh suspension or solution.

In the second period (after 18 days), this operation was repeated every 24 hours in order to have a higher conversion and, therefore, a more accurate analysis. This operation was repeated about 8 times, so that the new medium could replace the previous one by about 90% and a steady-state could be reached. Therefore, about 1 liter of the various feed solutions or suspensions were prepared and stored at 4°C at a time.

When fresh solution was fed into the reactors, the solution was shaken in order to have a homogeneous feed. Table 12 lists the composition of the solutions used successively in the first two runs. Solution 1 was used only to start a viable culture. Solutions 1, 2 and 3 were sterilized.

A third run was done at 37°C to confirm the original results using only four of the original eight solutions. This run was started again with Postgate's media, but on the fourth day a media of acid mine water and yeast extract was used. On the 16th day, digester sludge was added to the acid mine water-yeast extract feed. Finally, on the 21st day, the yeast extract was removed and only acid mine water, digester sludge, and some Na_2S to keep the oxidation potential down were used. The compositions of the successive feed solutions for run 3 are given in Table 13.

8.3 Results

The results are shown in Figures 10 and 11. In runs 1 and 2, solutions 2 and 3 gave good results, although they do not compare with the results of continuous runs with the lactate medium. If the addition of fresh nutrient is averaged over the day, the average dilution ratio is 0.5 $days^{-1}$, that is, 0.021 $hours^{-1}$. In continuous runs with the lactate medium, the sulfate conversion was much higher for similar conditions.

The use of digester sludge provoked a reduction in conversion and the substitution of the artificial solution with acid mine water was (with solution 5) so unfavorable, at first, that the dilution ratio had to be halved in order to have a significant conversion. Solution 6, however, showed a progressive adaptation to acid mine water and digester sludge.

Table 12 - Media for Semibatch Runs 1 and 2

Solution	1	2	3	4	5	6	7	8
NH_4Cl	500	500	500	500	-	-	-	-*
KH_2PO_4	250	250	250	250	-	-	-	-*
Na_2SO_4	2250	2250	2250	2250	-	-	-	-*
$MgSO_4 \cdot 7H_2O$	30	30	30	30	-	-	-	-*
$CaCl_2 \cdot 6H_2O$	30	30	30	30	-	-	-	-*
Na lactate	3000	1500	-	-	-	-	-	-*
Yeast extract	500	3000	3000	3000	3000	3000	-	-*
Na citrate$\cdot 2H_2O$	150	150	150	150	-	-	-	-*
$FeSO_4 \cdot 7H_2O$	2	2	2	2	-	-	-	-*
Norton AMW	-	-	-	-	1	1	1	1**
Digester sludge	-	-	-	yes	4	4	4	4**
Na_2S	yes	yes	yes	yes	yes	-	-	-

* Composition in parts per million

**Ratio of Acid Mine Water to Digester Sludge in Feed

Table 13. Media for Semibatch Run 3

Solution	1(Postgate's)	2	3	4
Na lactate	3000	-	-	-*
Sulfate Ion ($SO_4^=$)	1500	2420	1920	1920*
Yeast Extract	3000	3000	3000	-*
Norton Acid Mine Water	-	1	1	1**
Digester Sludge	-	-	4	4**
Sodium Hydroxide	-	yes	-	
Na_2S	yes	yes	yes	yes
pH	7.0	7.2	7.4	6.3

* Composition in parts per million

**Ratio of Acid Mine Water to Digester Sludge

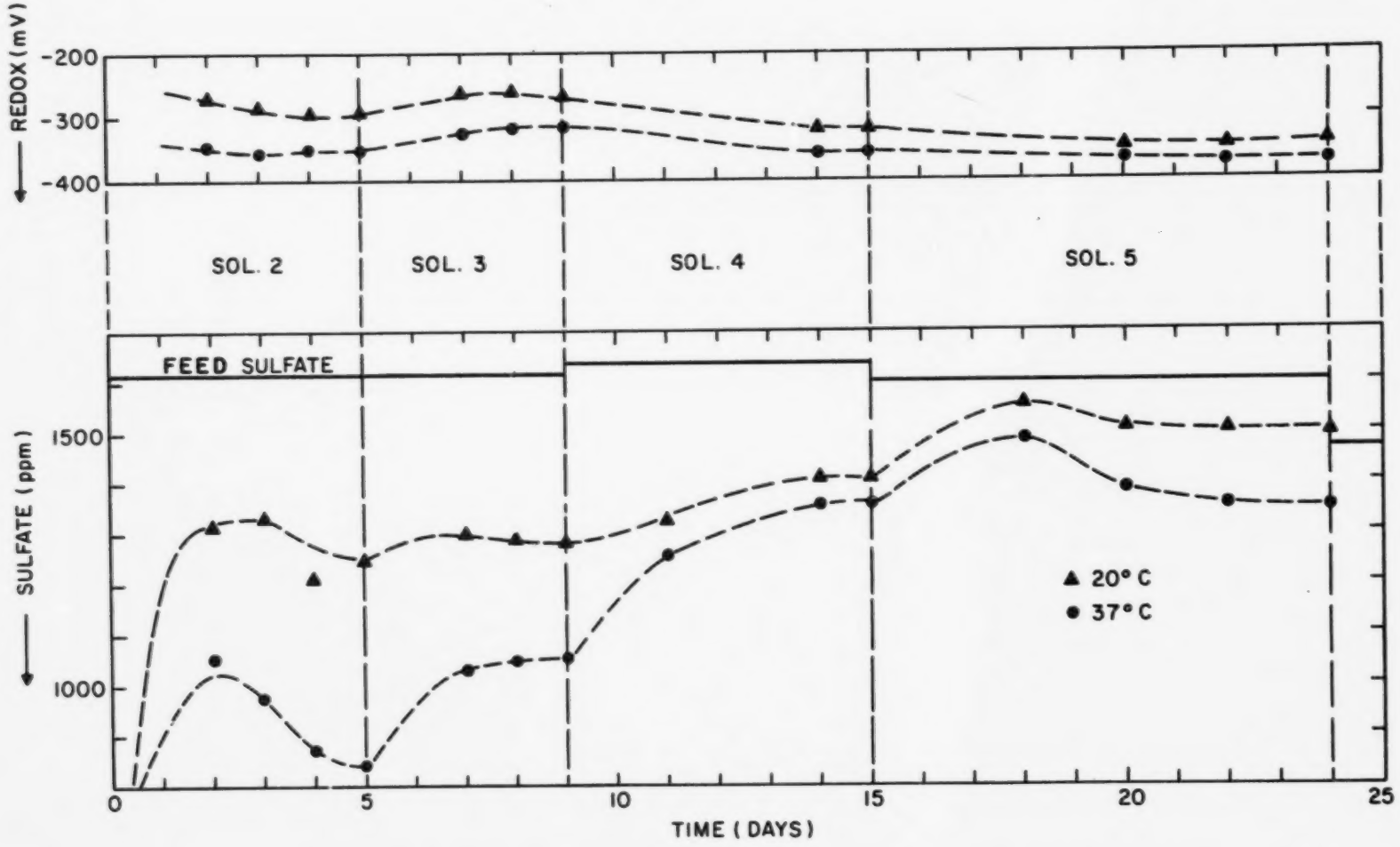


FIGURE 10 SULFATE REDUCTION AND REDOX POTENTIAL IN SEMIBATCH RUN I

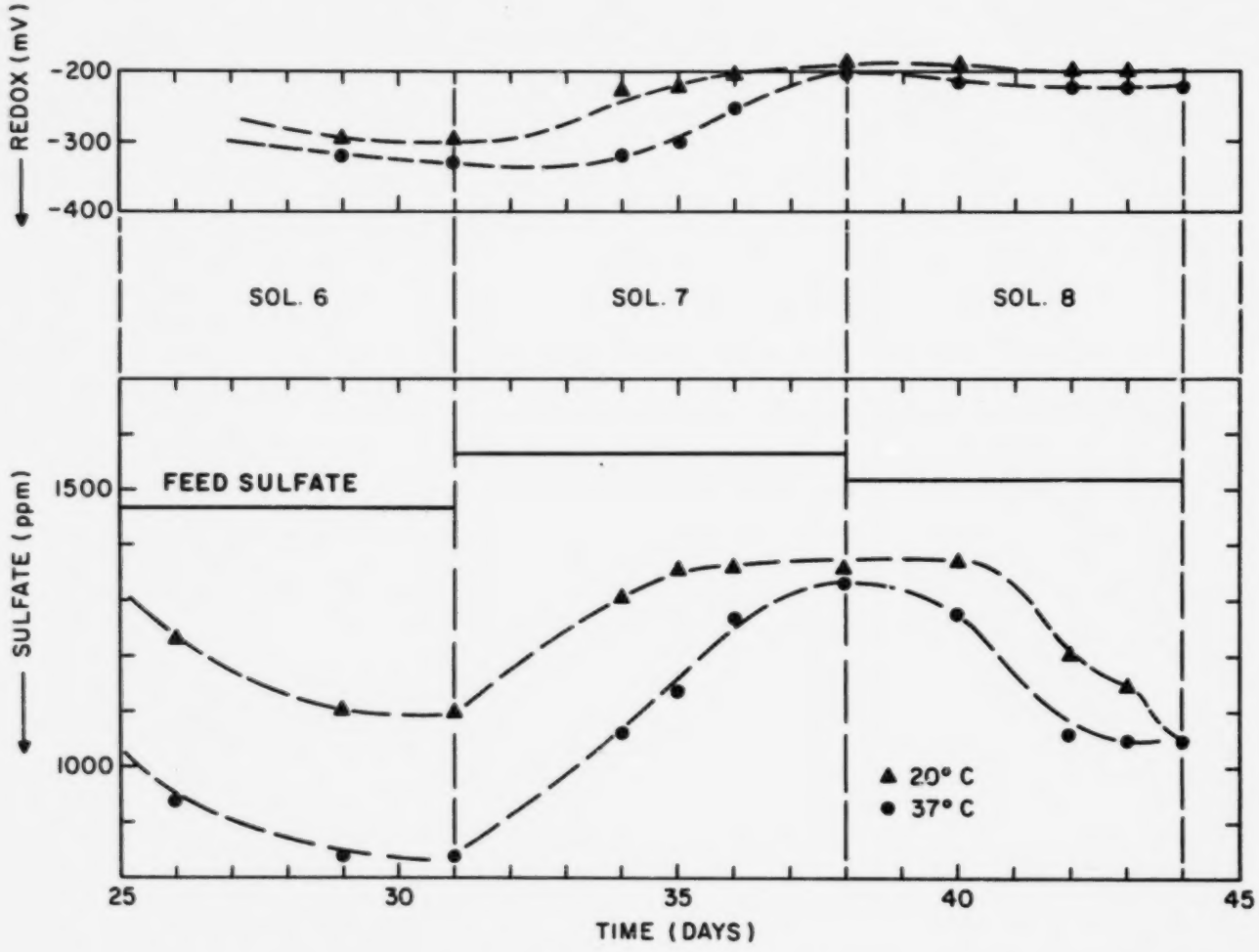


FIGURE 10 SULFATE REDUCTION AND REDOX POTENTIAL IN SEMIBATCH RUN I (CONTINUED)

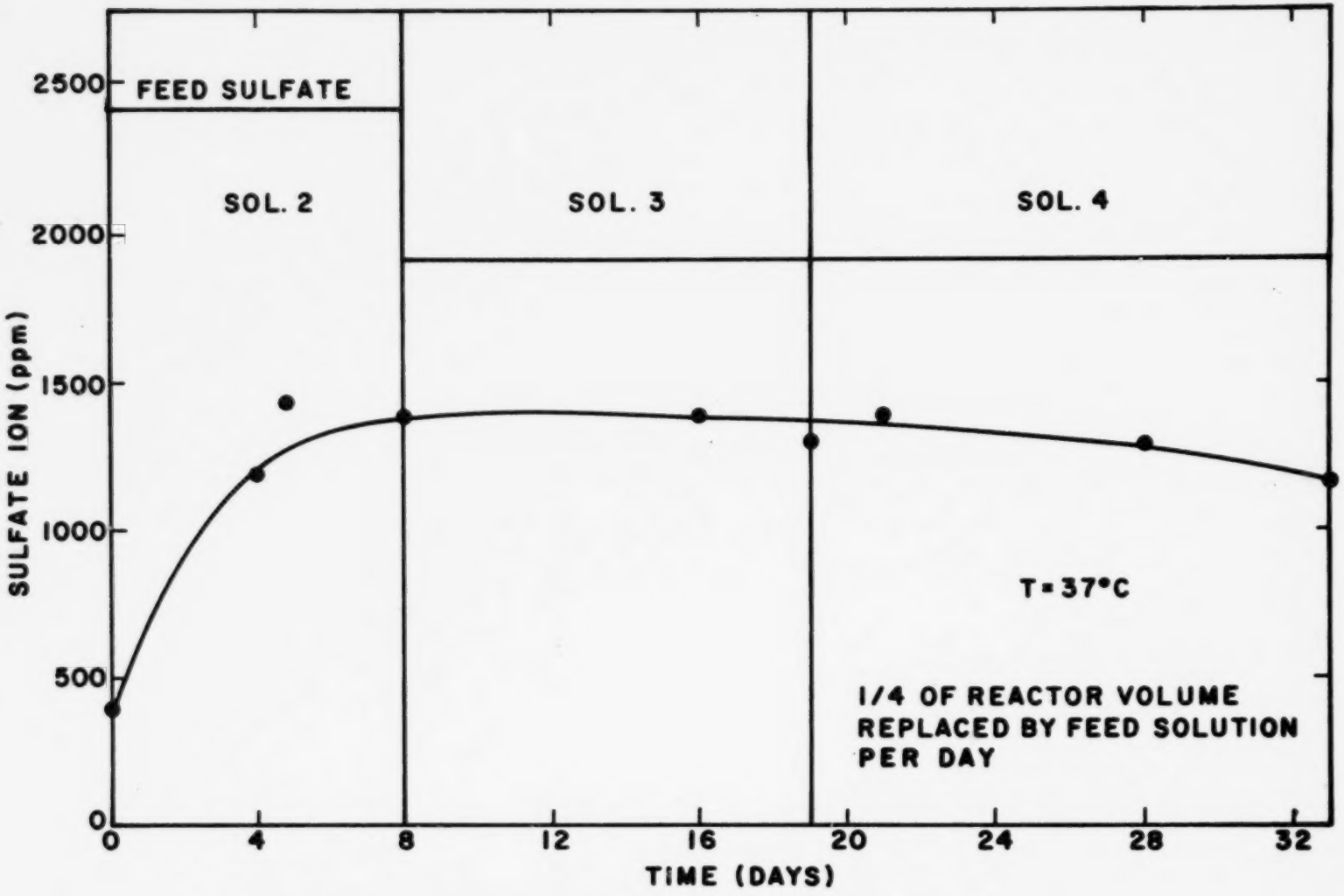


FIGURE II SULFATE REDUCTION IN SEMIBATCH RUN 3

Solutions 7 and 8 were prepared by mixing the same raw materials and the slight difference of initial sulfate concentration is probably due only to experimental errors in sampling and mixing acid mine water and digester sludge. Also, in this case, the first reaction was unfavorable, but in the succeeding days bacteria were able to develop a viable culture in solution 8.

On the 43rd day, the solution at 20°C was kept for one day at 37°C and sulfate concentration, redox potential and pH became equal to the corresponding parameters of the solution that was kept at 37°C for the entire run. On the 44th day, the semibatch run was stopped and the two solutions were left to react at 37°C as batch runs; the final sulfate concentration was 890 ppm after 14 days for both solutions.

The lower sulfate reduction rate with sludge and acid mine water compared to lactate media is probably largely due to the lower concentration of organic carbon. This ranges 0.200 to 0.400 gr/l in filtered sludge although it will be considerably higher in unfiltered sludge, whereas it is about 1.3 gr/l in undiluted Postgate media. For example, if the total feed organic carbon concentration is 0.6 gr/liter, with an average dilution ratio of 0.0105 $hr.^{-1}$, and the rate expression determined from the continuous lactate runs is used (section 7, p. 54):

$$0.33\ C = D,$$

or

$$C = 0.032 \text{ gr/liter}$$

Thus, $\Delta C = 0.6 - 0.032 = 0.568$ gr/liter, and if the moles of carbon necessary to reduce one mole of sulfate is the same in the sludge as it is in lactate media (6 moles organic carbon for one mole of sulfate), the total amount of sulfate reduced (ΔS) should be

$$\Delta S = \frac{6R}{96}\ C = 0.425 \text{ gr/}\ell\ ,$$

quite close to the 0.47 gr/ℓ observed at 37°C in the semibatch reactor using only digester sludge and acid mine water. This similarity implies that the rate-controlling mechanisms involved in sulfate reduction in acid mine water and sludge are the same as with lactate media. This conclusion should be further checked, however, by additional continuous runs with sludge and acid mine water.

Similar results were obtained in the third run. After the substitution of acid mine water for Postgate media, there was a rise in sulfate concentration, but a substantial reduction in sulfate ion still occurred. This persisted as digester sludge was added and when the yeast extract was removed from the feed solutions. The final sulfate reduction with acid mine water and digester sludge is consistently higher (0.72 gr/ℓ vs 0.47 gr/ℓ) than observed in the first run. This may be due to the more favorable redox potential (-360 mv vs - 200 mv) or higher organic carbon content in the sludge.

8.4 Discussion

The results of these runs prove that D. Desulfuricans can be progressively adapted to a complex medium made by acid mine water and digester sludge alone.

In the first run, the low absolute value of the final redox potential indicates that the culture is barely viable and this can explain the contradictory results obtained from batch runs; probably a small variation of the parameters can make the culture unstable and under apparently similar conditions, there could be either a significant reduction or no reduction at all. If the redox potential is kept sufficiently low, the sulfate reduction is improved and probably the culture is more stable.

9. CONTINUOUS CULTURE

9.1 Experimental Procedure

The continuous culture system used in this work was the chemostat described below.

9.1.1 Apparatus

Figure 12 is a flow chart of a continuous system employed in this work. The reactor was a two liter round bottom pyrex flask which was kept in a constant temperature bath with the temperature controlled within 0.1°C. For runs below room temperature, chilled water was circulated in a coil. The temperature of the water in the bath was controlled by a Precision Electronic Relay.

Stirring in the reactor is achieved by a Teflon-coated magnetic bar. The liquid level is set by adjusting a platinum probe to the desired level and controlling the influent pump by a Thermo-cap on-off controller.

Fresh medium was fed to the reactor and the effluent was pumped out by two Variable Speed Masterflex Tubing Pumps. The effluent rate was kept at the desired rate, while the feed was controlled by the on-off level controller. The system was kept at a partial pressure of about one-half atmosphere with a Dyna-Vac Vacuum Pump. The hydrogen sulfide and CO_2 produced were evacuated continuously and vented to the hood. All lines used were silicone tubing. They were connected with glass tubing and sealed with silicone seal to prevent the possible contamination from other organisms.

The entire system was sterilized by autoclaving at 15 psig for 20 minutes before it was put into place.

9.1.2 Procedure

About six liters of medium were prepared and the pH was adjusted to the value desired by adding 0.1 N NaOH or 0.1 N HCℓ. About two grams of Na_2S were added to reduce the oxidation potential of the solution below -200 mV. When the lactate medium was used, it was sterilized by autoclaving for 20 minutes at 15 psig. A final adjustment of pH and redox potential was needed occasionally after sterilization. The aforementioned chemicals were added aseptically, but without sterilization.

The medium was pumped into the reactor and the reactor was inoculated with about 50 ml of a grown culture of D. Desulfuricans. The continuous culture was started with a batch culture. After the batch culture in the reactor grew to a sufficiently high cell concentration to indicate that the exponential growth had begun. the continuous culture was then started. The effluent rate was set at a dilution ratio of about 0.06 - 0.09 h^{-1}. The cell concentration, sulfate concentration, redox potential and pH were monitored several times until the steady-state was reached.

After the steady-state concentration of sulfate and other

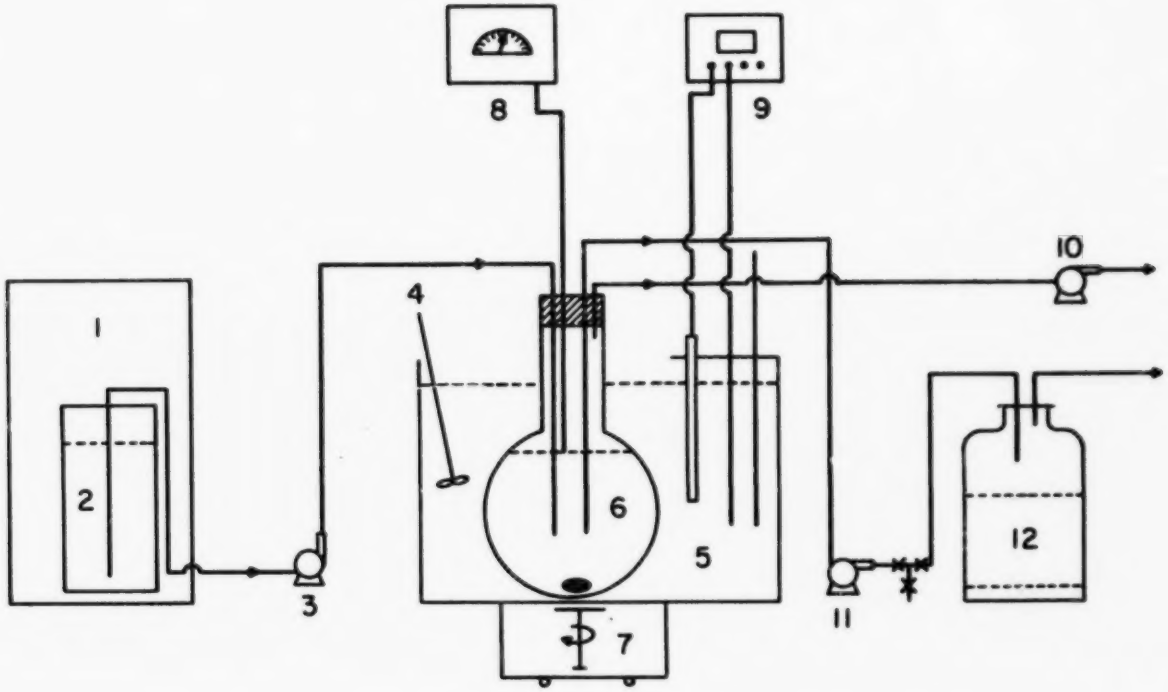


FIGURE 12. CHEMOSTAT FOR CONTINUOUS CULTURE

1. REFRIGERATOR; 2. NUTRIENT MEDIUM STORAGE TANK;
3., 11. MASTERFLEX TUBING PUMPS; 4. STIRRER;
5. CONSTANT TEMPERATURE BATH; 6. CULTIVATOR;
7. MAGNETIC STIRRER; 8. LEVEL CONTROLLER;
9. TEMPERATURE CONTROLLER; 10. DYNAC VACUUM PUMP;
12. SPENT MEDIUM STORAGE TANK.

parameters had been monitored, the experiment proceeded to another set of operating conditions.

9.2 Runs with Lactate Medium at pH 7

9.2.1 Discussion of the Results

A set of runs was made with the influent pH at about 7, but at different temperatures and dilution ratios. The results are summarized in Figure 13, in which sulfate reduction is plotted against dilution ratios, and in Table 14 as well.

In order to interpret the results, sulfate concentration was used as a measure of substrate concentration. Even though lactate seems to be the most effective nutrient and the controlling substrate, analytical determination of lactate is not necessarily meaningful because yeast extract and sodium citrate can be used by D. Desulfuricans as organic substrate, as shown by batch experiments. This can lead to substantial errors, especially at high conversions, when the concentration of lactate becomes critically low and the conversion of yeast extract and sodium citrate becomes relatively important.

Figure 14 reports the Lineweaver-Burk plot of reciprocals of dilution ratio and effluent sulfate concentration at 37°C. The plots were analyzed with the least squares method. The constants of Monod equation can be evaluated, as the reciprocals of absolute values of intercepts of the line on both axis.

At 37°C, the equations are:

$$\frac{1}{\bar{\mu}} = \frac{\cdot}{D} = 4.06 \quad \frac{1}{S} + 0.957$$

From these two equations the following constants can be computed:

$$\bar{\mu} = 1.045 \ h^{-1}$$

$$K_s = 4.25 \ \ gr/\ell$$

Small errors in measurements of the dilution ratio or the sulfate concentration could also shift markedly the intercept of the line, changing significantly the value of $\bar{\mu}$. See also Appendix VIII and Appendix IX for accuracy of sulfate determination and flow control.

Analysis with the least squares method of Lineweaver-Burk plot at 18 and 10°C is not satisfactory*. At low conversion and dilution ratios, the possibility of substantial experimental error is greatly increased. In fact, the data appear to be too scattered and, therefore, it is difficult to draw meaningful conclusions.

The linear interpolation shows a value of K_s of 4.25 g/ℓ that is substantially greater than S, especially at low dilution ratios.

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*See Figure 15.

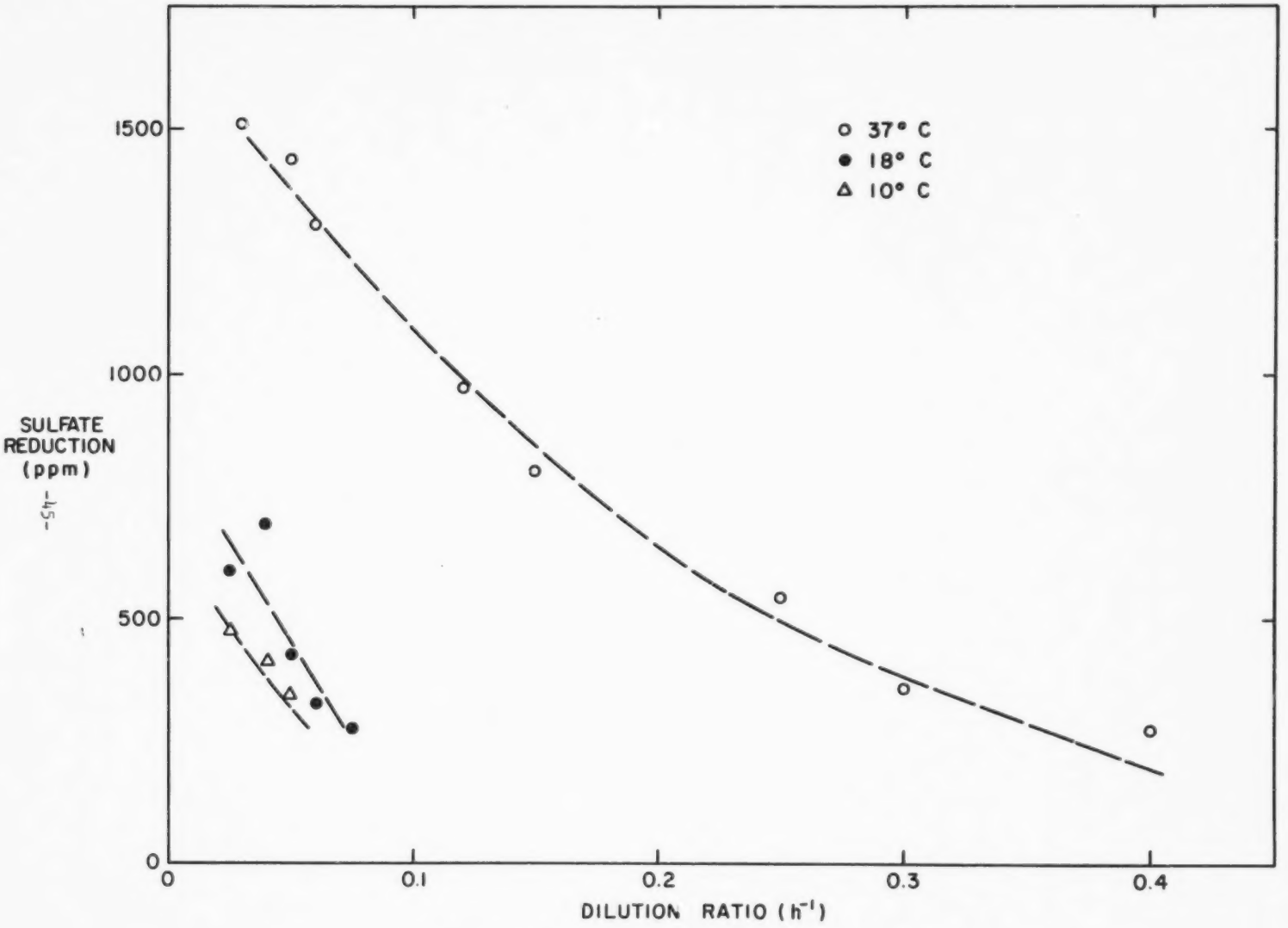


FIGURE 13 SULFATE REDUCTION vs DILUTION RATIO

Table 14 - Data of Continuous Runs

Temperature (°C)	Dilution Ratio (h^{-1})	Influent SO_4^{--} (ppm)	Effluent SO_4^{--} (ppm)	SO_4^{--} uptake (ppm)
37	0.03	1640	130	1510
37	0.05	1620	180	1440
37	0.06	1640	330	1310
37	0.12	1470	680	790
37	0.15	1660	860	800
37	0.25	1620	1150	370
37	0.30	1560	1200	360
37	0.40	1560	1280	280
18	0.025	1640	1040	600
18	0.04	1620	920	700
18	0.05	1590	1170	420
18	0.06	1640	1320	320
18	0.075	1640	1360	280
10	0.025	1620	1140	480
10	0.04	1580	1160	420
10	0.05	1620	1280	340

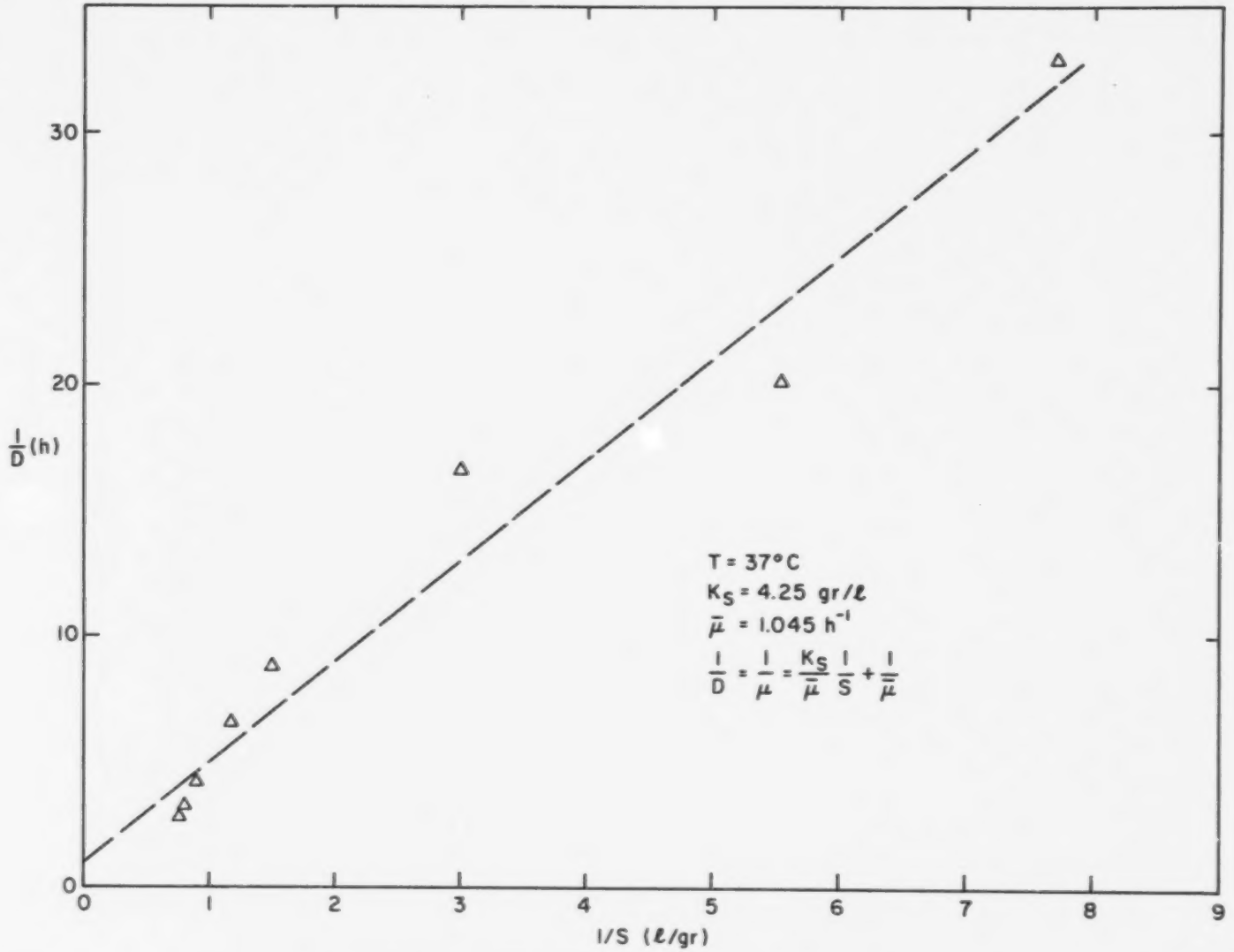


FIGURE 14 LINEWEAVER-BURK PLOT 1/D vs 1/S

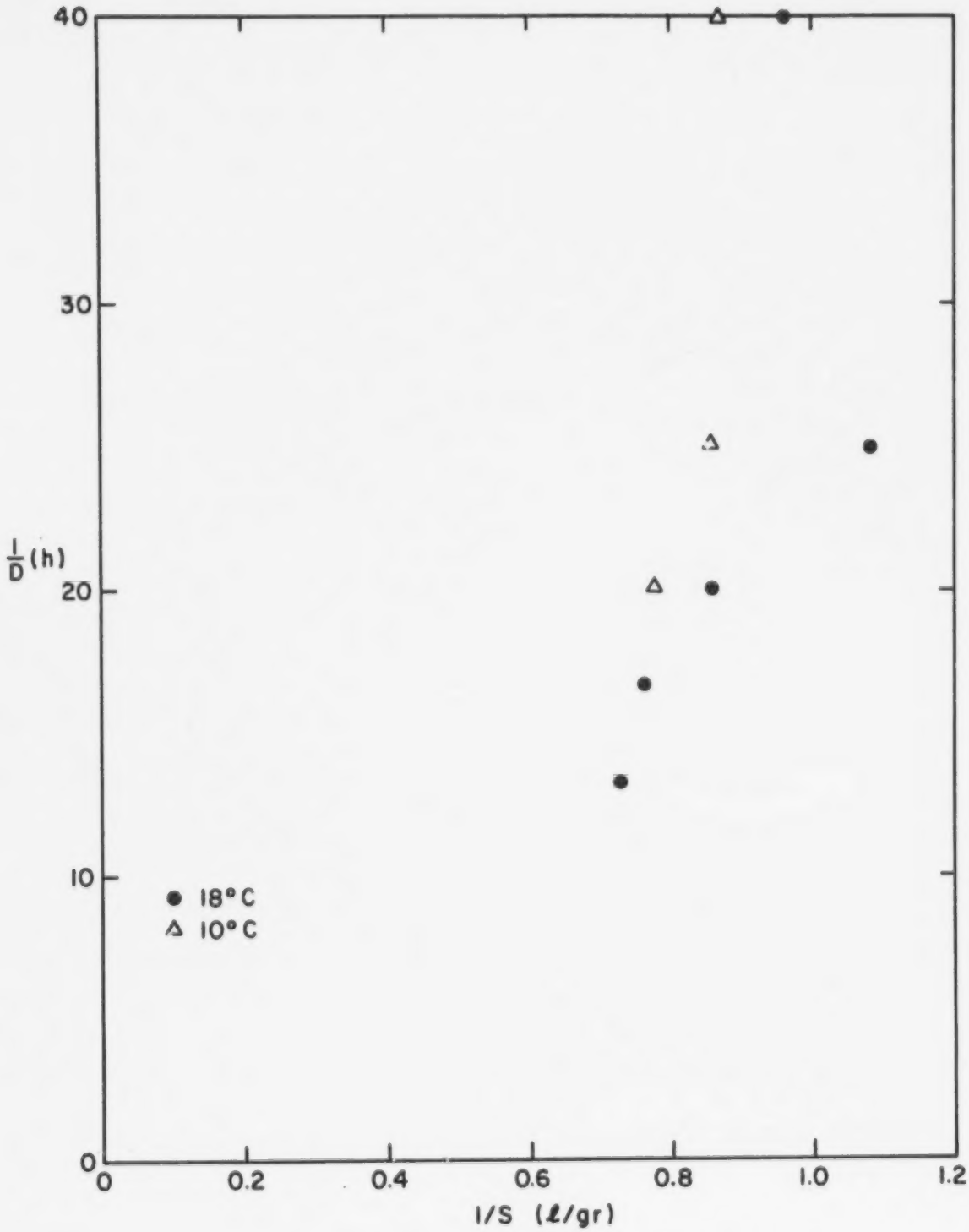


FIGURE 15 LINEWEAVER-BURK PLOT OF 1/D vs 1/S

Therefore, the linear approach as shown in equation 4.3.5.2 was tried.

$$D = \frac{\bar{\mu}}{K_s} S$$

Figure 16 illustrates this approach.

The linear relationship is evident for $D > 0.15$ (h^{-1}) at 37°C. The slope of the line is about 0.180 ℓ/h.gr, which is not too different from the slope obtained by dividing $\bar{\mu}/K_s$ obtained from linear interpolation, that is, $\bar{\mu}/K_s = 0.245$ ℓ/h.gr.

9.2.2 Influence of the Wall Reaction

The second part of the curve shows a different slope, and the shape of the whole curve is concave, instead of convex (the second derivative is positive instead of negative).

This phenomenon can be explained by noting that the reaction is taking place on the wall of the reactor. In this case, equation 4.3.5.9 should apply. Figure 17 shows the relationship between $D(S_o - S)$ and S.

Figure 18 shows the Lineweaver-Burk plot of equation 4.3.5.7. The coefficient can be found by a least squares analysis. The values are:

$$Q_w = 0.152 \text{ gr/ℓ.h}$$

$$K_w = 0.271 \text{ gr/ℓ}$$

In this case, K_w is smaller than S at low conversions (high dilution ratio). Therefore, equation 4.3.5.7 can be written as

$$D(S_o - S) = \frac{Q_w S}{K_w + S} \approx Q_w$$

The existence of a wall reaction can explain some irregular behavior, since it can act to an unpredicatable extent, because it is difficult to reproduce the local conditions that allow the bacterial culture to stick on the wall or to fall off, and a systematic study of this phenomenon was beyond the purposes of this study. Therefore, the wall reaction approach should be considered more as a qualitative explanation of some unexpected behavior, rather than as a quantitative analysis. Figure 19 shows the relationship between $1/D(S_o - S)$ and $1/S$ at 10°C and 18°C, but the data are too scattered for a meaningfull conclusion.

9.2.3 Comparison with Hsu's Experiments

In Figure 16 the data of Hsu also are reported.

The behavior is different at low and high dilution ratios. At high dilution ratios, wash-out occurred and the reaction on the wall probably did not take place. At low dilution ratios, the conversion

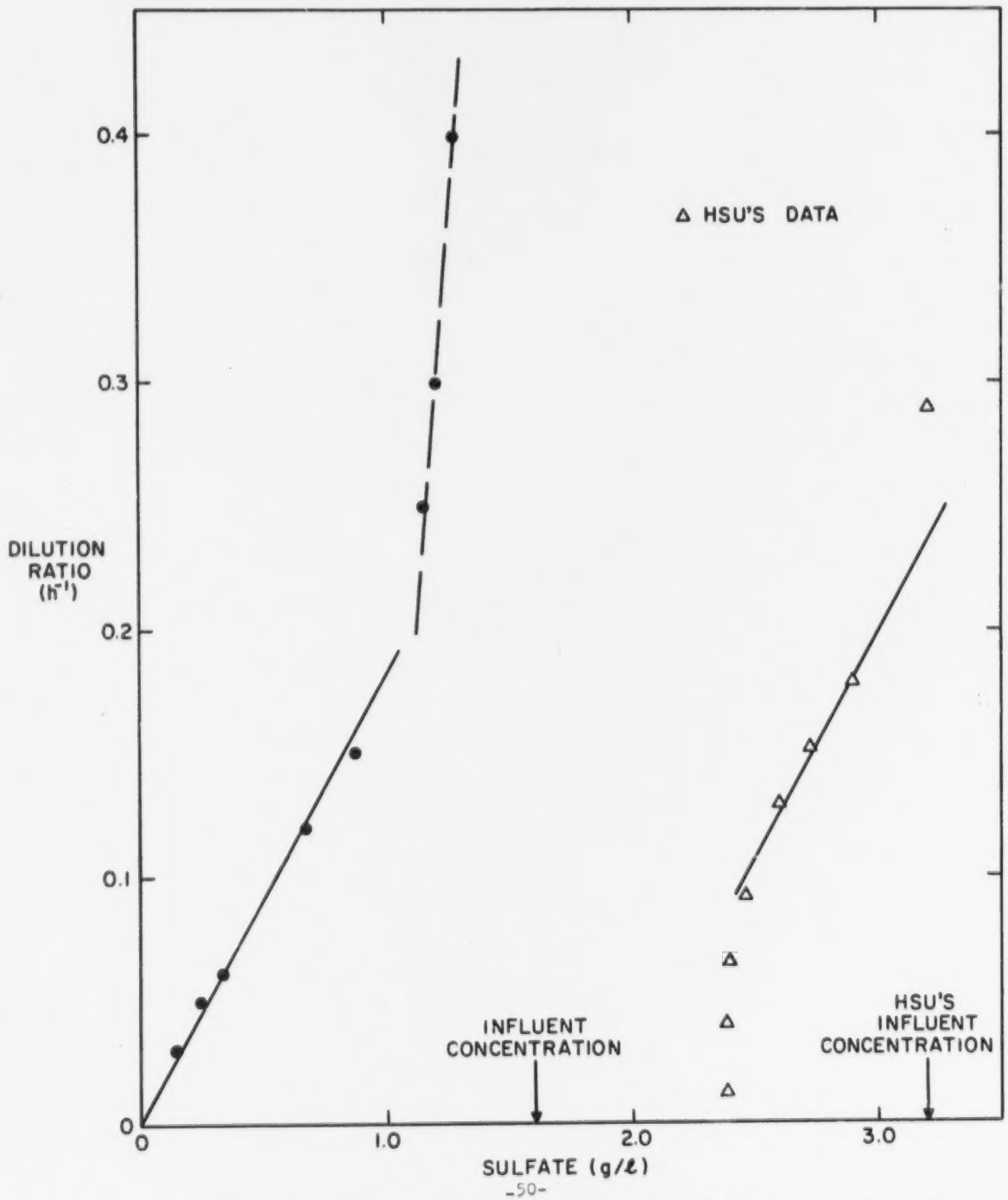


FIGURE 16 DILUTION RATIO vs EFFLUENT SULPHATE CONCENTRATION

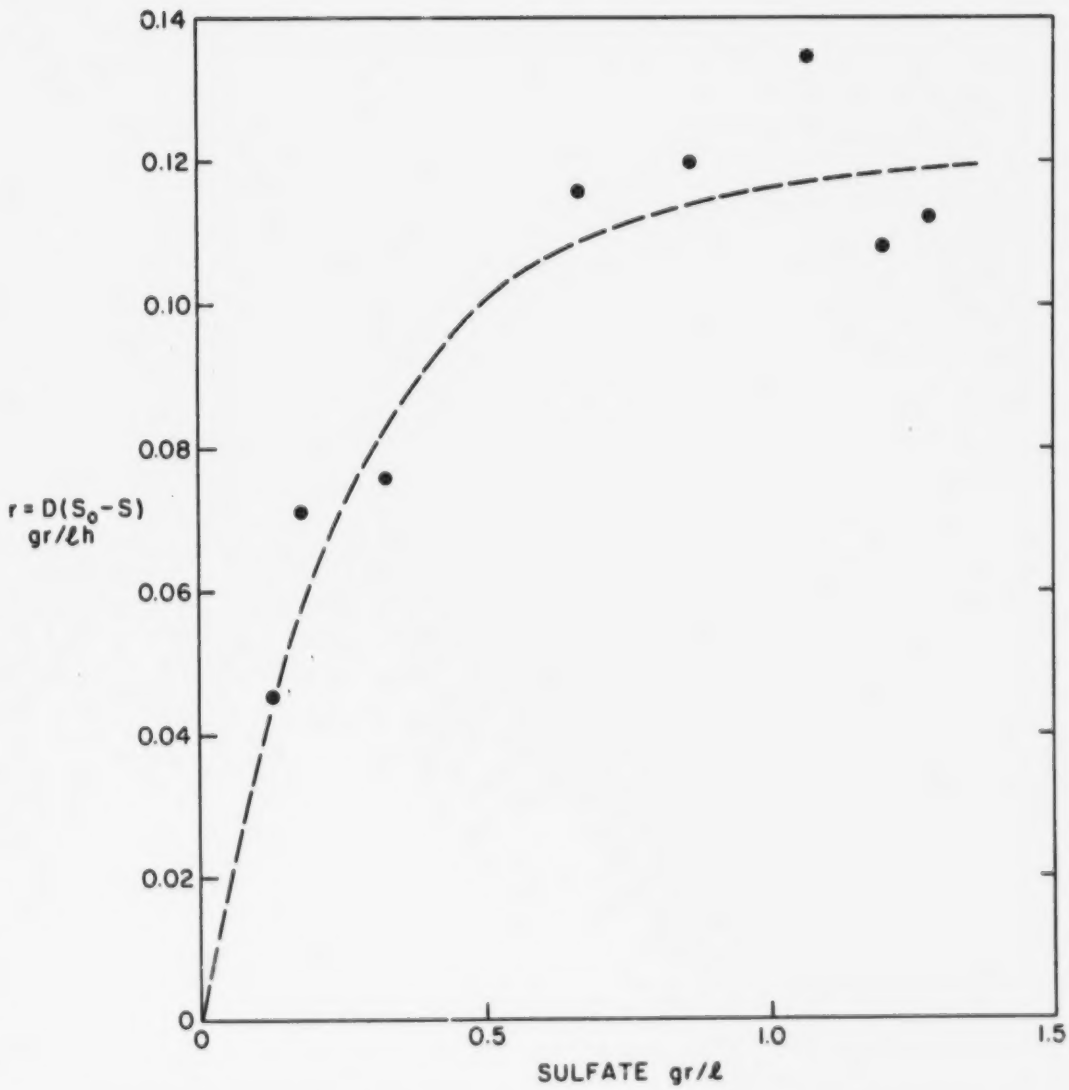


FIGURE 17 RATE OF SULFATE REDUCTION vs EFFLUENT SULFATE CONCENTRATION

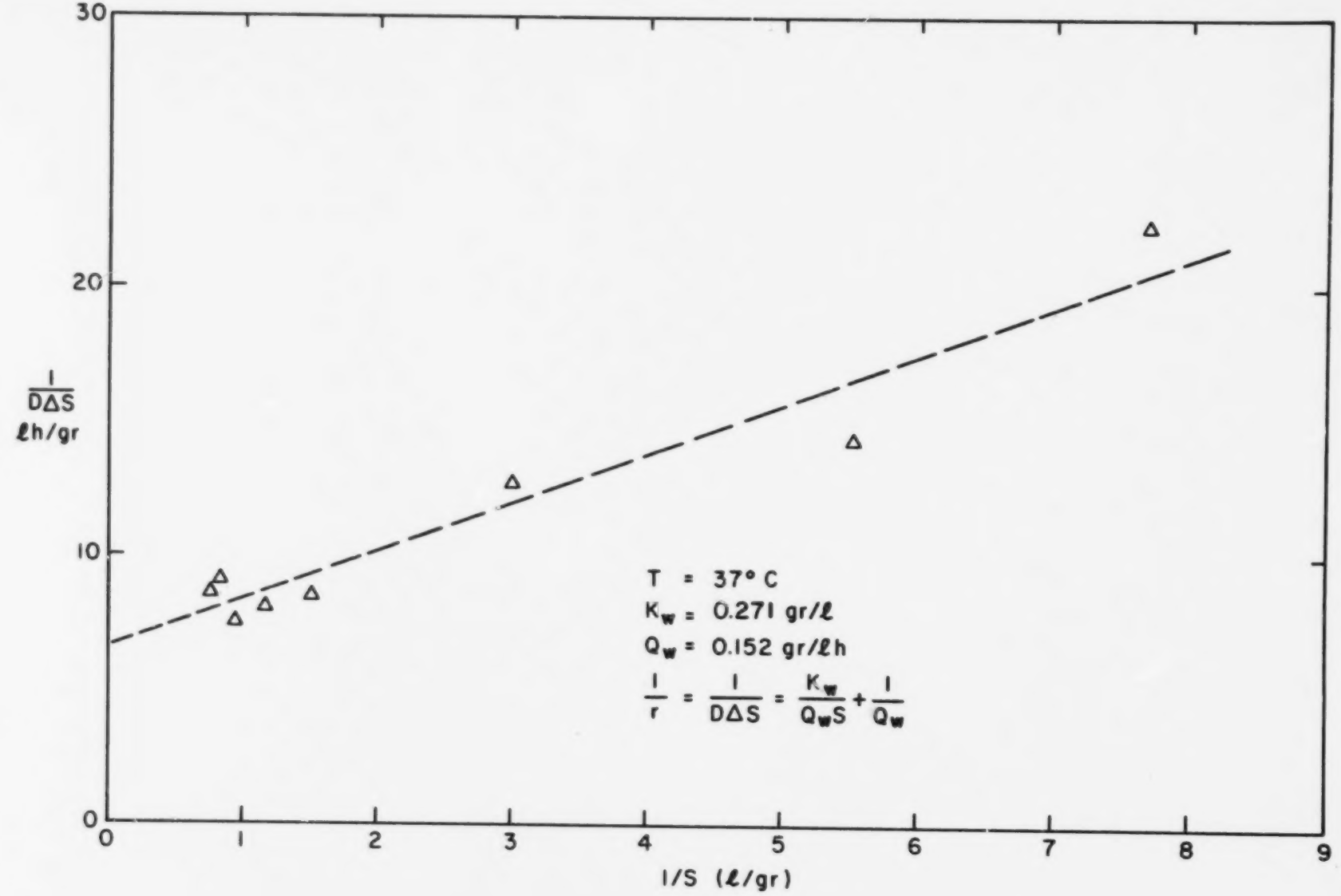


FIGURE 18 LINEWEAVER-BURK PLOT OF 1/r vs 1/S

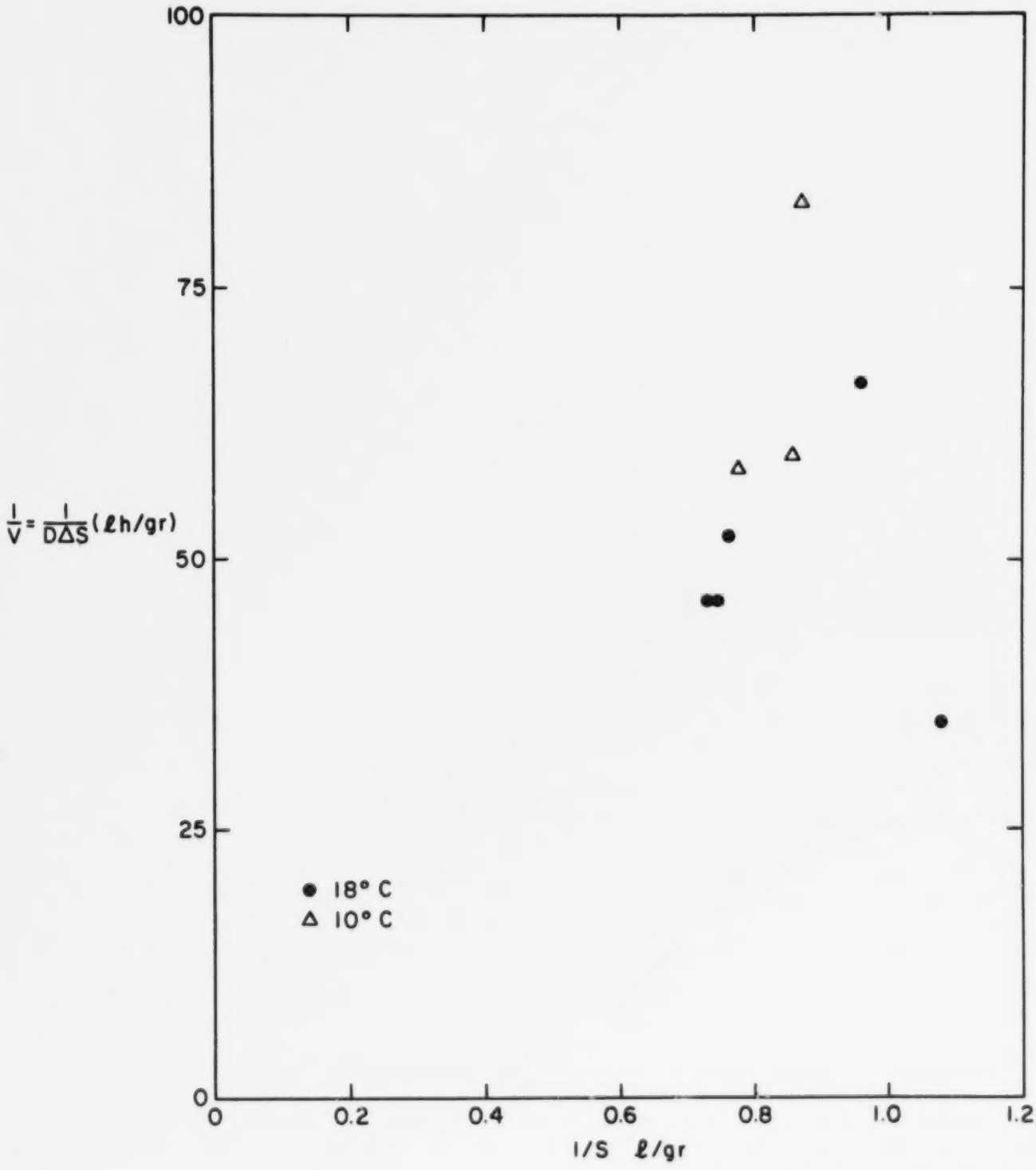


FIGURE 19 LINEWEAVER-BURK PLOT OF 1/r vs 1/S

of SO_4^{--} was limited by the low initial lactate content. In fact, he used about 2.54 g/ℓ of lactate with an influent SO_4^{--} of 3.2 g/ℓ. If all lactate were converted, the maximum SO_4^{--} uptake would be = 1.080 gr/ℓ, with a minimum effluent of 2.12 g/ℓ SO_4^{--}, which is not too far from the measured 2.39 g/ℓ. This means that at dilution ratios lower than 0.0645 h^{-1} the conversion in Hsu's experiments may have been reduced by the low lactate/sulfate ratio. In fact, with a higher lactate-sulfate initial ratio, the reaction proceeds almost to the complete reduction of sulfate. At medium dilution ratios, not many data are available but the slope of the two curves look very similar.

Since Hsu's experiments were carried out at a different range lactate/sulfate ratio, the sulfate reduction rates should be related to the organic substrates concentration, because yeast extract and sodium citrate might contribute to the reaction as well as lactate. Figure 20 shows the relationship between organic carbon content and various initial concentrations of organic components.

Figure 21 is taken from batch experiments and shows the relationship between initial organic carbon concentration and sulfate reduction.

Assuming that all initial organic carbon is consumed by sulfate reduction, it is possible to correlate sulfate reduction to organic carbon consumption and to derive from Figures 13, 20 and 21 a relationship between the dilution ratio and the organic carbon effluent.

Figure 22 shows the relationship between D and effluent organic carbon.

If the linear approach is used, in the case of organic carbon, it is possible to write:

$$D = \frac{\bar{\mu}}{K_s} C$$

The slope of the line in Figure 22 is 0.33 ℓ/gr.h at 37°C. In Figure 22 the data of Hsu are also reported following the same procedure.

Again, Hsu's data show a lower conversion at corresponding dilution ratios, but the slope of the line drawn through some central points is about the same. It is possible that the conversion is depressed for two reasons:

- non-optimal ratio sulfate/organic carbon
- bacteria unable to fully utilize yeast extract and sodium citrate as organic substrates

In fact, assuming that only lactate can be a suitable substrate, the curve is shifted to the left and almost superimposes the curve D vs C at 37°C as shown in Figure 22.

Exact comparison with Hsu's results cannot be expected, however, because, as shown in the semibatch run, D. Desulfuricans

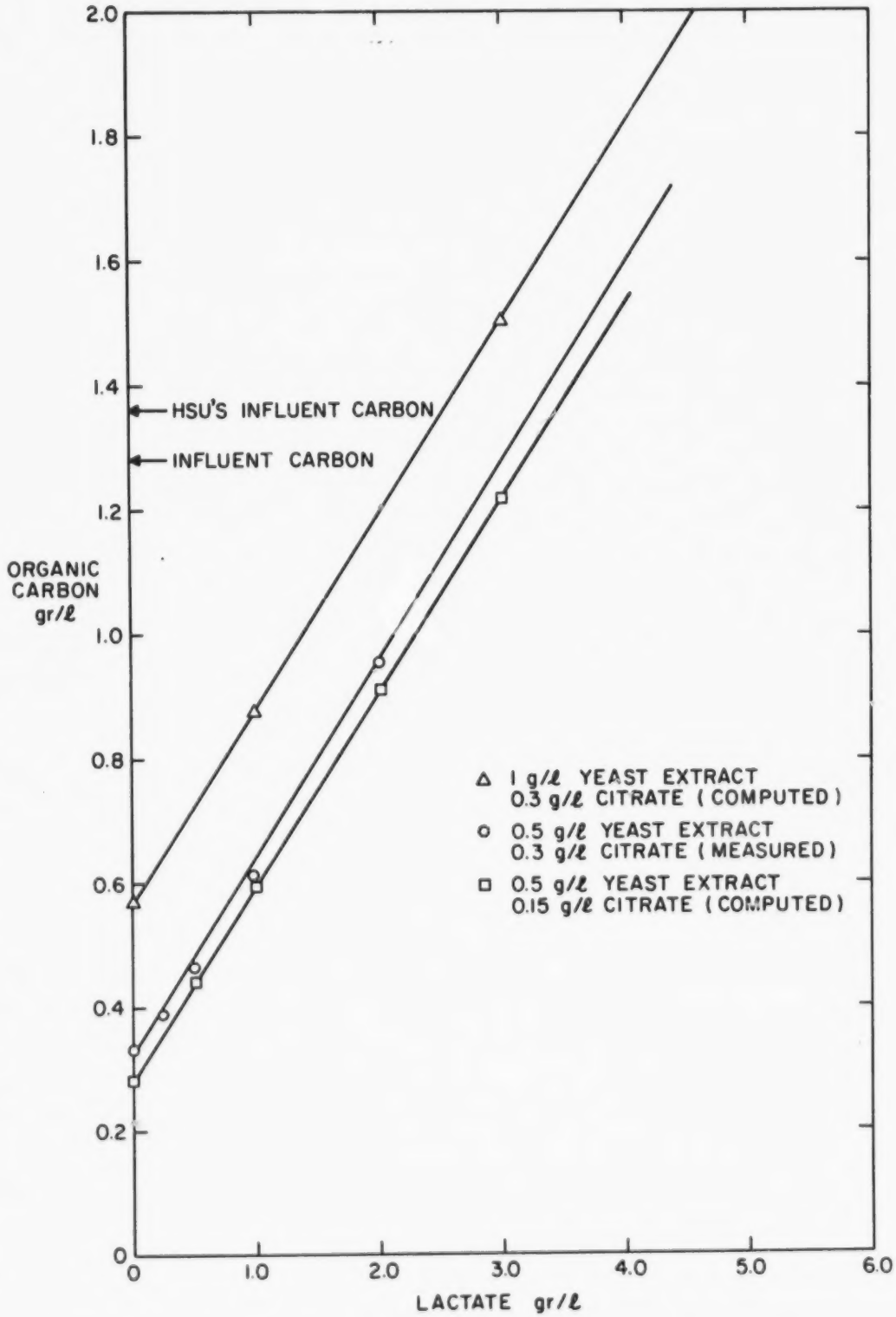


FIGURE 20 ORGANIC CARBON CONCENTRATION OF DIFFERENT MODIFIED POSTGATE MEDIA

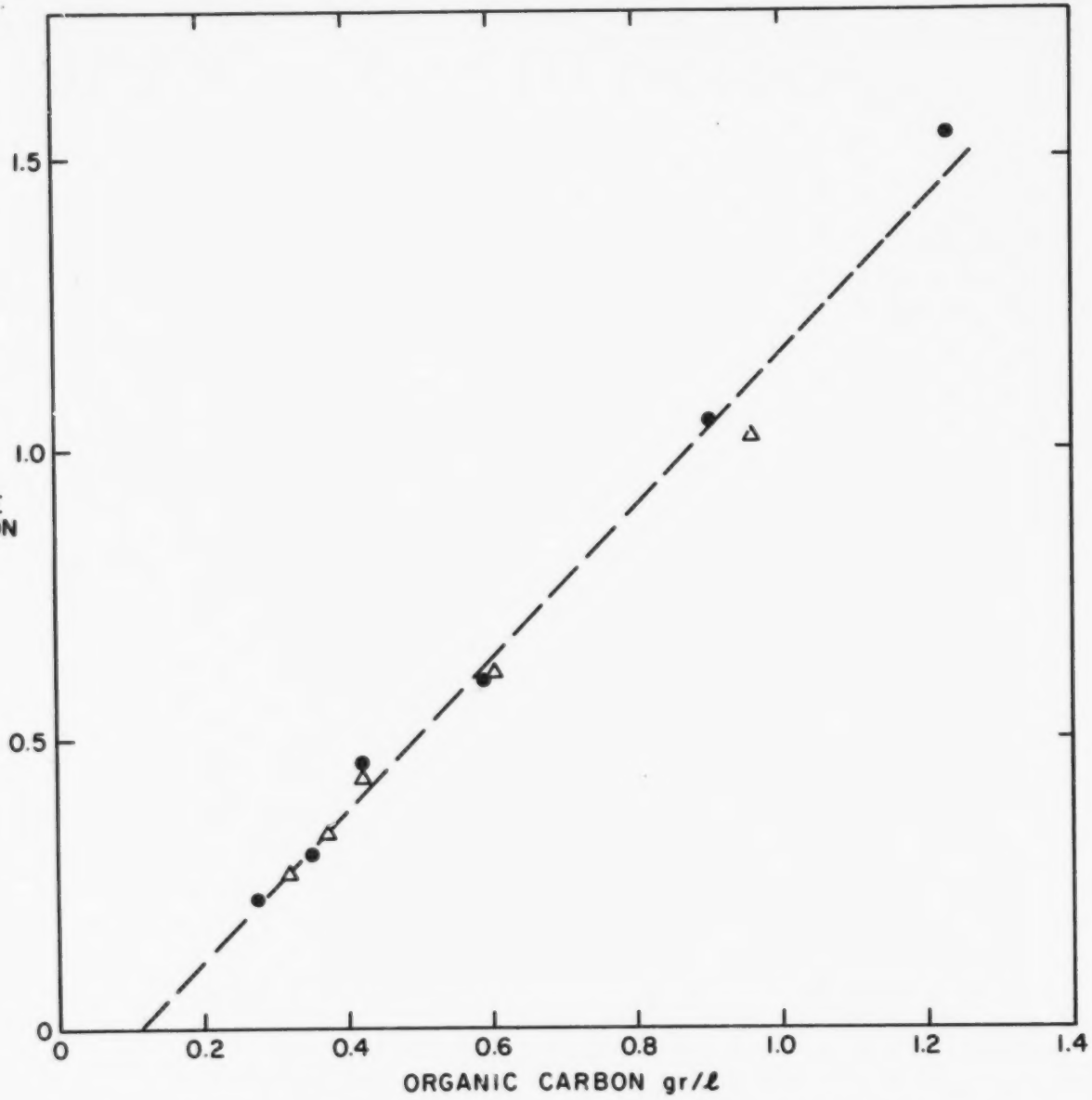


FIGURE 21 SULFATE REDUCTION vs ORGANIC CARBON AVAILABLE

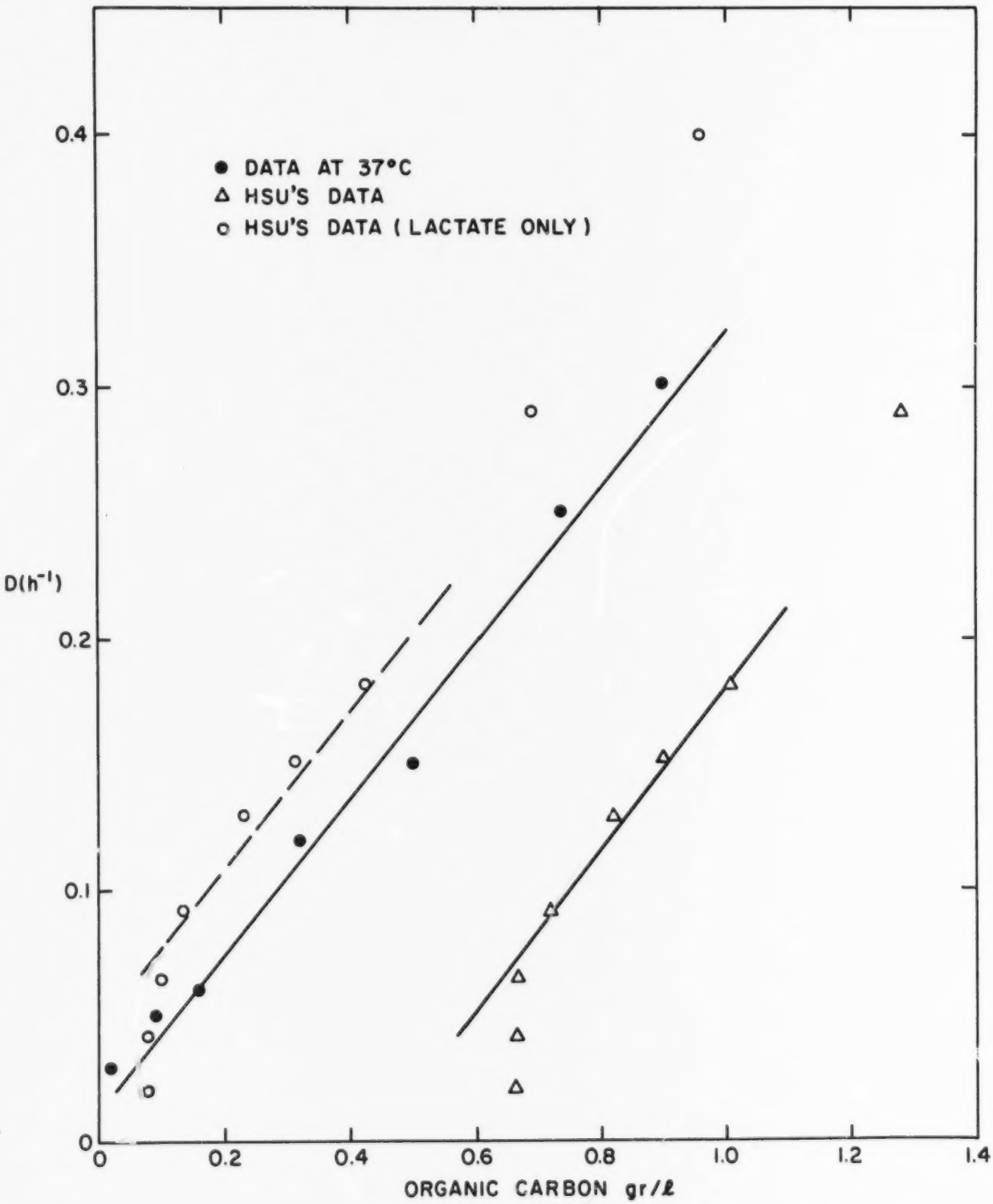


FIGURE 22 DILUTION RATIO vs EFFLUENT ORGANIC CARBON

can be adapted to the medium. Hsu's culture might not have adapted to the lactate medium in the same way as the culture here.

In this study, the bacteria were grown for many generations in the lactate medium; therefore, the higher sulfate reduction can be due, also, to adaptation.

9.2.4 Data at 18 and 10°C

The same approach as 9.2.1 can be used for 18 and 20°C. The data are plotted in Figure 23.

The data are too scattered for a significant analysis, because, in this case, sulfate reduction is a result of a difference of two measures that have the same order of magnitude and, therefore, the error of analysis plays an important role.

Also, operation at very low dilution ratios is rather difficult and inaccurate, because the pumps work at their minimum flow rate. A line can be drawn in any case, either from the origin through the points or through the experimental points alone. The slope of the two lines at 18°C ranges from about 0.066 ℓ/gr.h to about 0.08 ℓ/g.h which is about 1/4 to 1/5 of the corresponding slope at 37°C.

The points at 10°C are too scattered to allow meaningful conclusions. However, a line with about the same slope as at 18°C can be drawn through the experimental points.

9.3 Yield

In our case, yield can be defined as:

$$Y_{x/s} = - \Delta S/\Delta X$$

sulfate reduced per unit increase in dry weight of cell concentration

that is, the reciprocal of $Y_{x/s}$ as defined in Chapter 4.3.3.

Figure 24 shows data collected in different runs, with different dilution ratios, influent pH and temperatures. All the data can be roughly connected with a straight line. The yield can be determined by measuring the slope of the line.

A least squares analysis gave the values

$$\Delta S = - 1,334 + 7.92\ \Delta X$$

Therefore,

$$Y_{x/s} = 7.92$$

The line does not pass through the origin of the two axis; there could be several reasons for this behavior:

- inorganic preciptated salts, like FeS, are retained by the filter

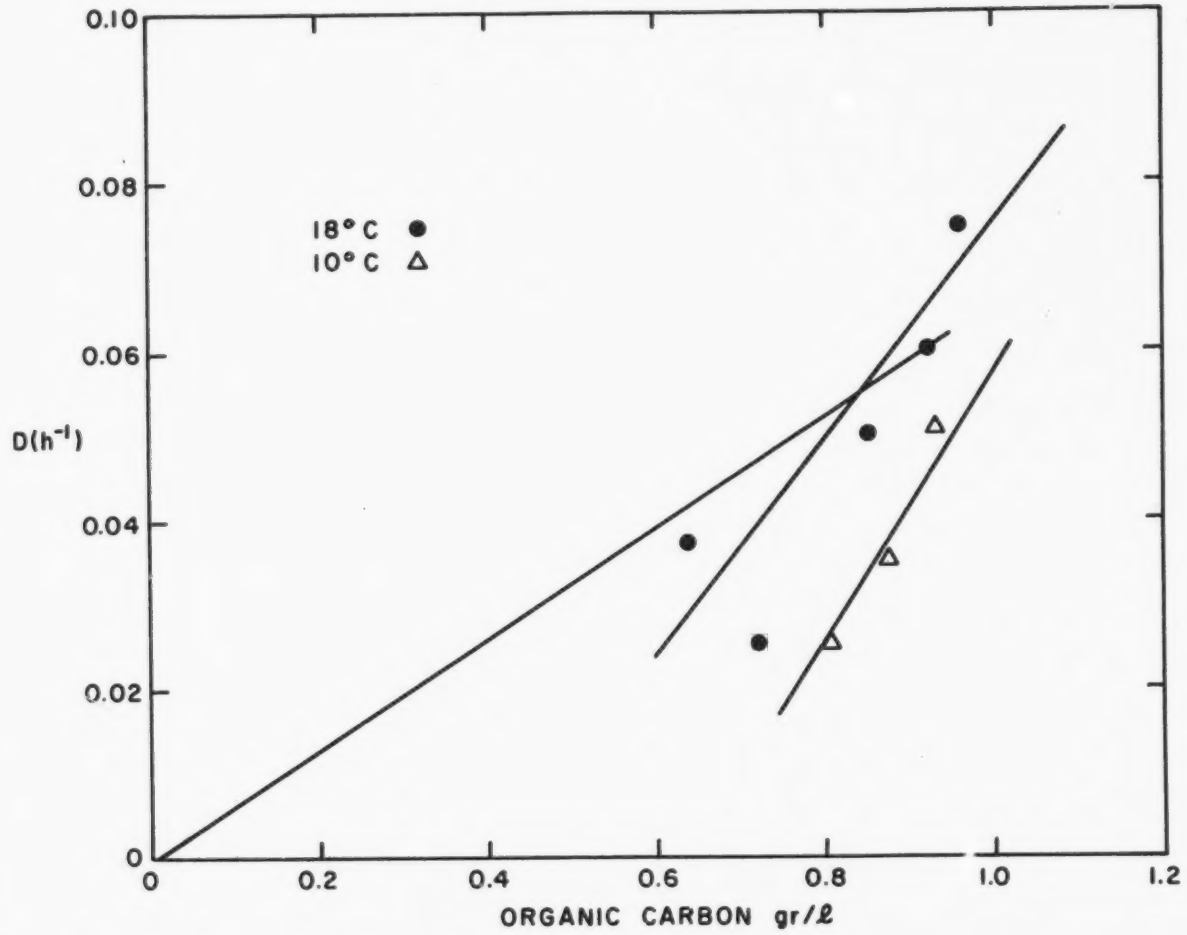


FIGURE 23 DILUTION RATIO vs EFFLUENT ORGANIC CARBON CONCENTRATION

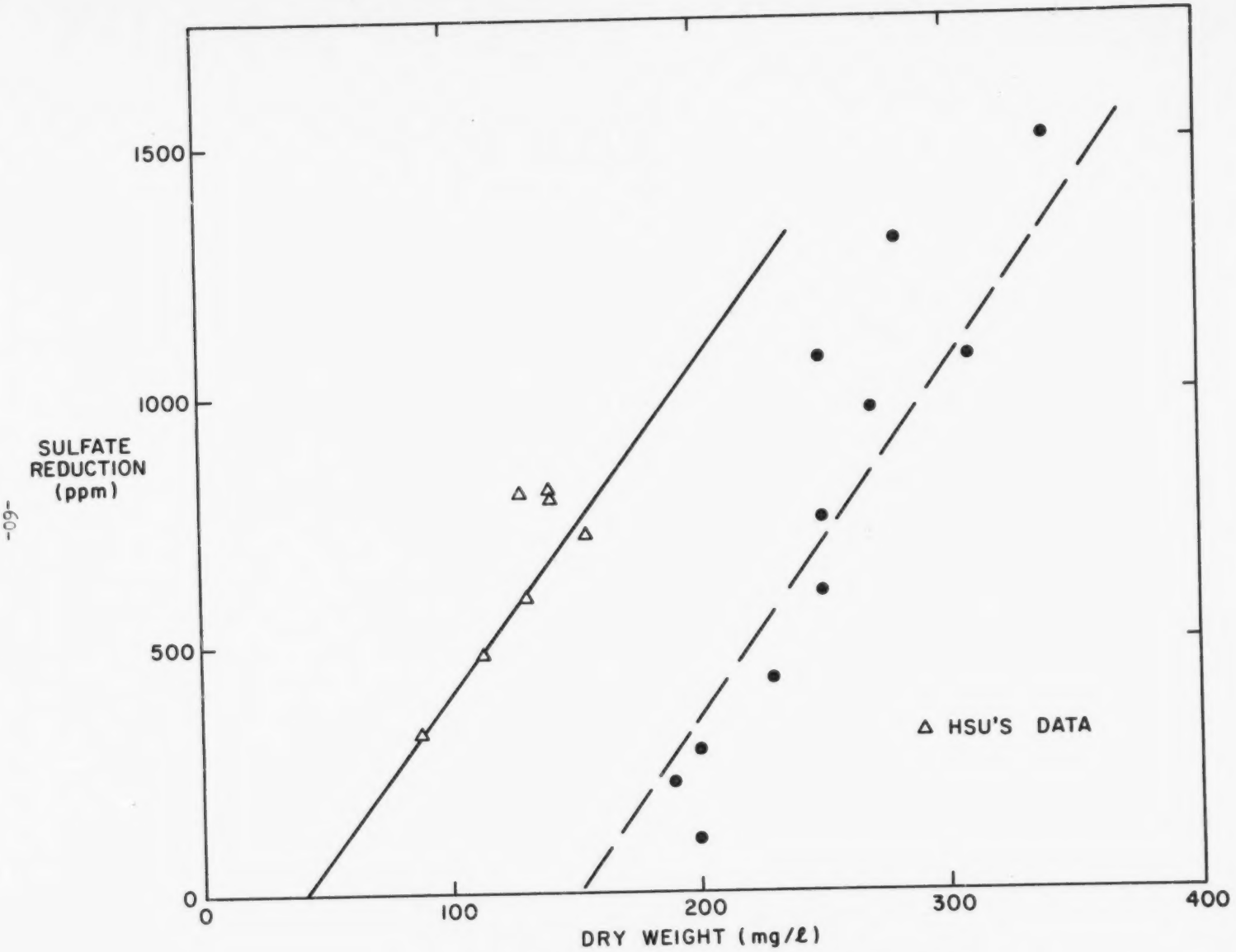


FIGURE 24 SULFATE REDUCTION vs DRY WEIGHT

- organic and inorganic salts retained by the biomass
- detachment of biomass from the wall of the reactor

In addition, in Figure 24, the yield coefficient obtained by Hsu is plotted. The slope is essentially the same. However, the two lines do not coincide. This can be due to the fact that Hsu used only traces of $FeSO_4$, and, therefore, there was no precipitate of FeS. Moreover, he used a Millipore filter with openings of 0.22 ± 0.02 instead of a Seitz filter with an average pore size of 0.01.

9.4 Influent pH Effect

The Postgate medium at half concentration (as shown in Appendix II), was used and a continuous culture with an influent pH of 7 was first established. Then at constant dilution ratio (0.09 h^{-1}) and constant temperature (37°C) the influent pH was lowered in steps, after the continuous culture reached a steady-state in each step. The results are shown in Figure 25.

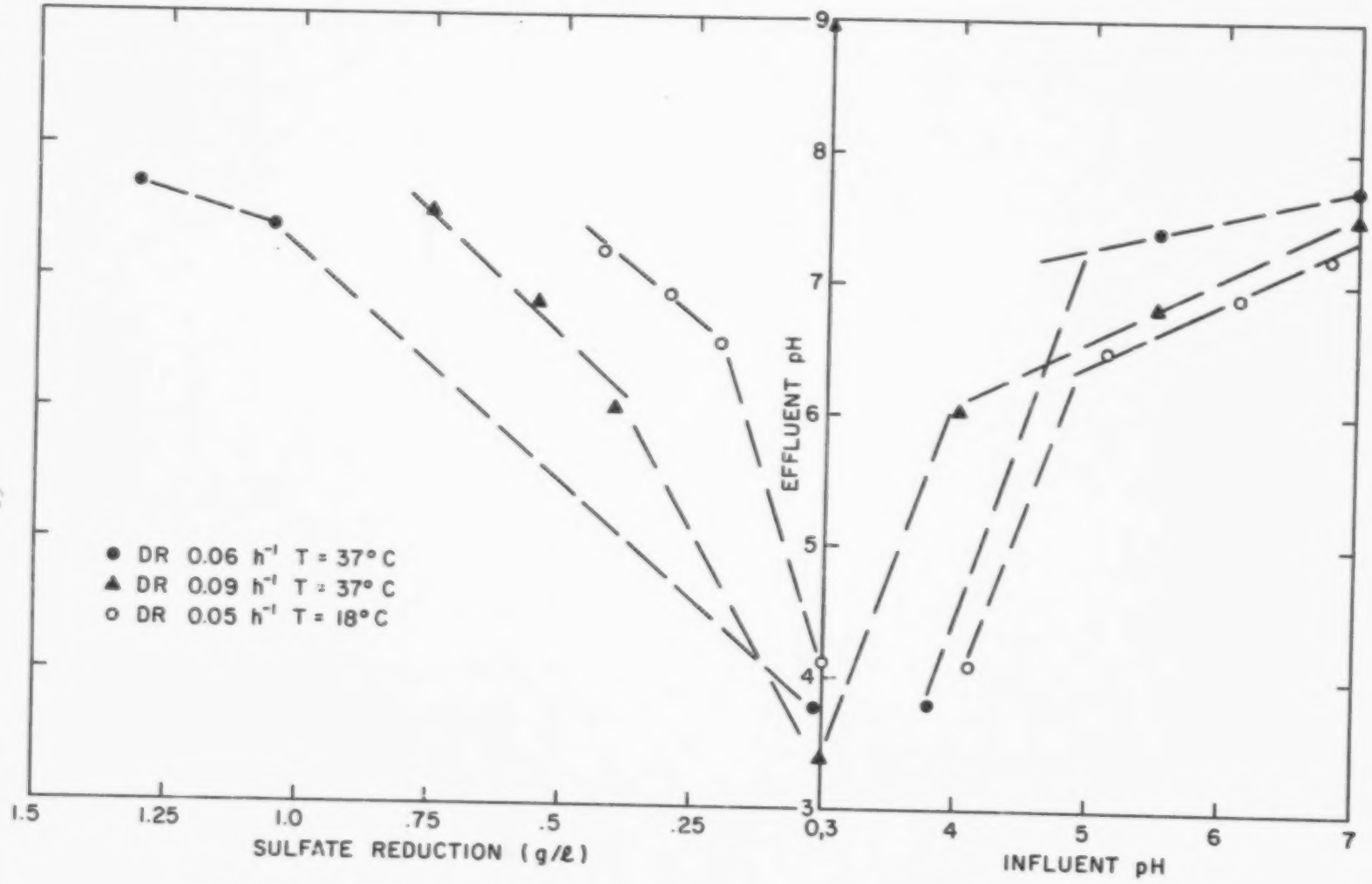
A viable culture with significant sulfate reduction occurred for an influent pH as low as 4. Below this pH the growth stopped. The critical pH for continuous culture seems to be the pH in the reactor. Growth proceeded as long as the reactor's pH remained at about 6.0 or above. When the influent pH dropped to 3, the reduction in acidity of the influent by the bacteria evidently was not sufficient to raise the pH of the culture to a viable level.

However, the influent pH which the culture can tolerate depends more on the basicity required to raise the pH of the influent pH to 6.0 or above, rather than on the influent pH itself, since the acidity must be neutralized in the reactor before a viable culture can be maintained. Figure 26 shows the neutralization curve of the influent solution in which pH was lowered to 2.85 with HCℓ 0.1 N. The acidity that must be neutralized to raise the pH of the medium from 4.0 to 6.0 is about 11 ml NaOH 0.1 N, for a 100 ml sample. This is equivalent to acidity of about 550 ppm of $CaCO_3$ from pH 4.0 to 6.0. On the other hand, to raise the pH from 3.0 to 6.0 would require about 34 ml NaOH 0.1 N for a sample of 100 ml. This corresponds to about 1700 ppm of $CaCO_3$.

The experiment was repeated at 37°C and dilution ratio of 0.06 h^{-1} and at 18°C and dilution ratio of 0.05 h^{-1}. The critical point seems to be at pH between 4.0 and 5.0. At pH higher than 5.0 every culture grew normally, while at pH 4.0 one culture grew and the other two stopped growing. This phenomenon could be explained by noting the abrupt change in slope of the acidity curve between pH 4.0 and 5.0. In fact, at pH 5.0 the slope is about 0.3 pH increase for 1 ml of NaOH added, while at pH 4.0 the slope is about one third of the above value. This means that at pH 4.0 a solution requires much more basic agent in order to be neutralized than a solution at pH 5.0.

9.5 Continuous Cultures with Sludge

Several continuous runs with medium shown in Appendix IV with no lactate, but with digester sludge as an organic substrate were made at



SULFATE REDUCTION vs EFFLUENT pH

EFFLUENT pH vs INFLUENT pH

FIGURE 25

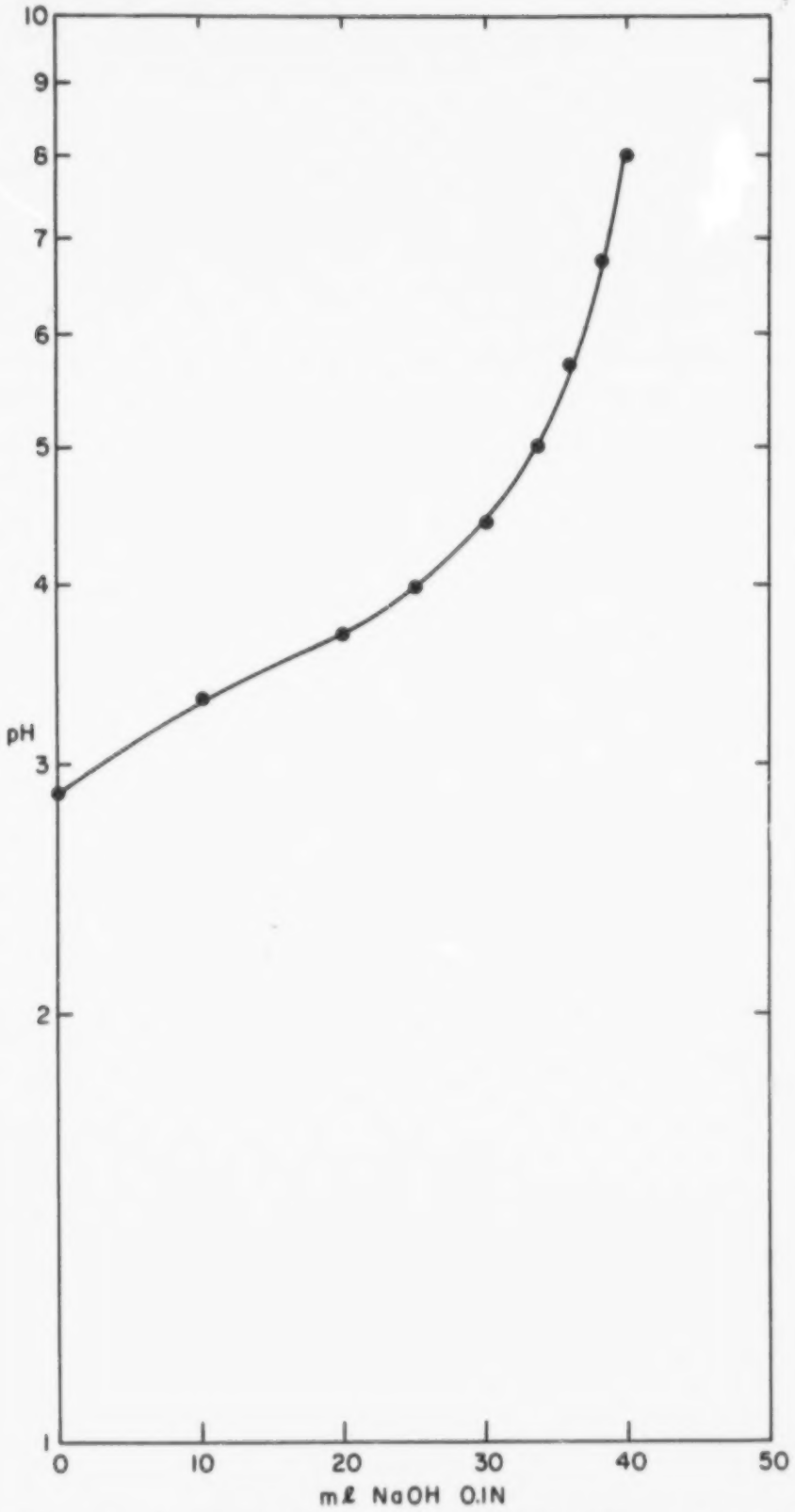


FIGURE 26 ACIDITY CURVE OF POSTGATE MEDIUM (100 mℓ)

37°C and 25°C with an influent pH 5.5 and 7.0. The solution was not sterilized, in this case, but was filtered in order to avoid the clogging of the tubes. A summary of the results is shown in Table 15.

Sulfate reduction was observed for the influent media with the higher organic carbon concentration. No appreciable reduction (above the normal error of analysis of about ± 0.1 gr/ℓ) was observed at 25°C and at 37°C with an influent pH 5.5.

Table 15 - Continuous Cultures with Sludge

Run	1	2	3	4
Temperature (°C)	37	25	37	37
Influent pH	7.0	7.0	7.0	5.5
Initial Organic Carbon (gr/ℓ)	0.185	0.185	0.485	0.205
Initial Sulfate (gr/ℓ)	2.98	2.98	3.05	2.80
Sulfate Reduction (gr/ℓ)	0.270	0.1	0.457	0.1
Dilution Ratio ($hr.^{-1}$)	0.03	0.03	0.03	0.03

The carbon content of the solution was always a little lower than expected; evidently, some organic carbon precipitates upon mixing the digester sludge with salts. No definite conclusion can be drawn from the experiment, because yeast extract and sodium citrate might have contributed to the reaction by an unpredictable extent.

NOMENCLATURE

D = dilution ratio, $hour^{-1}$

E,e = enzyme, enzyme concentration

$E\text{-}S,c$ = enzyme-substrate complex, enzyme-substrate concentration

k_i = reaction rate constants ($i = +1, -1, +2$)

K_m = the Michaelis-Menten constant

Q = maximum rate of production in enzyme-substrate reaction

P = product, product concentration

r = reaction rate

X = cell concentration

K_s = saturation constant

$Y_{i/j}$ = yield coefficient ($i=x,p$; $j=s,x$)

V = volume of the reactor

F = flow rate

S_o,S = initial and final sulfate concentration

W = reactor-solution interface

Q_w = maximum rate of reaction at the interface

K_w = reaction constant at the interface

C = organic carbon concentration

μ = specific growth rate

$\bar{\mu}$ = maximum specific growth rate when the substrate concentration is unlimited

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APPENDICES

APPENDIX I

Postgate Medium C

Yeast Extract	1.0 g/liter
KH_2PO_4	0.5 g/liter
NH_4Cl	1.0 g/liter
Na_2SO_4	4.5 g/liter
$CaCl_2 \cdot 6H_2O$	0.06 g/liter
$MgSO_4 \cdot 7H_2O$	0.06 g/liter
Sodium Lactate*	6.0 g/liter
Sodium Citrate$\cdot 2H_2O$	0.3 g/liter
$FeSO_4 \cdot 7H_2O$	0.1 g/liter

*Net weight of Sodium Lactate from a syrup 60% wt.

APPENDIX II

Modified Postgate Medium C

Yeast Extract	0.5	g/liter
KH_2PO_4	0.25	g/liter
NH_4Cl	0.5	g/liter
Na_2SO_4	2.25	g/liter
$CaCl_2 \cdot 6H_2O$	0.03	g/liter
$MgSO_4 \cdot 7H_2O$	0.03	g/liter
Sodium Lactate*	3.0	g/liter
Sodium Citrate $\cdot 2H_2O$	0.15	g/liter
$FeSO_4 \cdot 7H_2O$	0.002	g/liter

*Net weight of Sodium Lactate from a syrup 60% wt.

APPENDIX III

Modified Postgate Medium C

KH_2PO_4	0.25 g/liter
NH_4Cl	0.5 g/liter
$CaCl_2 \cdot 6H_2O$	0.03 g/liter
$MgSO_4 \cdot 7H_2O$	0.03 g/liter
Sodium Citrate$\cdot 2H_2O$	0.15 g/liter
$FeSO_4 \cdot 7H_2O$	0.002 g/liter

	Run 6.1.3	Run 6.1.4
Yeast Extract	variable	0.5 g/liter
Sodium Lactate*	3.0 g/liter	variable

*Net weight of Sodium Lactate from a syrup 60% wt.

APPENDIX IV

Digester Sludge Medium

(full concentration)

Yeast Extract	1.0	g/liter
KH_2PO_4	0.5	g/liter
Na_2SO_4	4.5	g/liter
$CaCl_2 \cdot 6H_2O$	0.06	g/liter
$MgSO_4 \cdot 7H_2O$	0.06	g/liter
Sodium Citrate$\cdot 2H_2O$	0.3	g/liter
$FeSO_4 \cdot 7H_2O$	0.002-0.005	g/liter
Digester Sludge		
Total Carbon	0.633-0.688	g/liter
Inorganic Carbon	0.385-0.431	g/liter
Organic Carbon	0.185-0.303	g/liter

APPENDIX V

Medium for Run 6.2.2*

Tube	1	2	3
Yeast Extract	0.25	0.50	1.0
KH_2PO_4	0.125	0.250	0.500
$NH_4C\ell$	0.25	0.50	1.00
$NaSO_4$	1.125	2.25	4.50
$CaC\ell_2 \cdot 6H_2O$	0.015	0.03	0.06
$MgSO_4 \cdot 7H_2O$	0.015	0.03	0.06
Sodium Citrate $\cdot 2H_2O$	0.075	0.15	0.30
$FeSO_4 \cdot 7H_2O$	0.0025	0.005	0.01
Digester Sludge			
Total Carbon	0.167	0.265	0.485
Inorganic Carbon	0.100	0.166	0.294
Organic Carbon	0.067	0.100	0.191

*Composition in grams per liter

APPENDIX VI

Media With Digester Sludge and Milk Whey*

Yeast Extract	0.5
KH_2PO_4	0.25
NH_4Cl	0.5
Na_2SO_4	1.8
$CaCl_2 \cdot 6H_2O$	0.03
$MgSO_4 \cdot 7H_2O$	0.03
Sodium·Citrate $2H_2O$	0.15
$FeSO_4 \cdot 7H_2O$	0.005

Run 6.2.2 in 1-liter flask

Digester Sludge:	Total Carbon	Inorganic Carbon	Organic Carbon
	0.682	0.316	0.366

Run 6.3.1 with Milk Whey

Tube	1	2	3	4	5
Total Carbon	0.557	0.981	1.950	3.515	5.840
Inorganic Carbon	0.177	0.150	0.130	0.120	0.100
Organic Carbon	0.380	0.831	1.820	3.395	5.740

Run 6.3.2	Tube	1	2	3	Run 6.3.3
Organic Carbon		0.25	0.5	1.0	4.5

*Composition in grams per liter

APPENDIX VII

Runs with Trastan and Sugar*

KH_2PO_4	0.25
NH_4Cl	0.5
Na_2SO_4	2.25
$CaCl_2 \cdot 6H_2O$	0.03
$MgSO_4 \cdot 7H_2O$	0.03
$FeSO_4 \cdot 7H_2O$	0.005

Run with Trastan

Total Carbon	3.050
Inorganic Carbon	0.050
Organic Carbon	3.000
Sulfate	3.840

Run with Sugar

	Tube 1	Tube 2
Organic Carbon	3.0	7.5

*Composition in gram per liter

APPENDIX VIII

Sulfate Determination-Turbidimetric Method (22)

A - Apparatus

- Magnetic stirrer
- Stop watch
- Spectrophotometer, for use at 420 mμ
- Measuring spoon: capacity of about 0.2 - 0.3 ml

B - Reagents

- Conditioning Reagent: mix 30 ml glycerol with a solution containing 30 ml conc. HCℓ, 300 ml distilled water, 100 ml 95% isopropyl alcohol and 75 grams of sodium chloride.
- Barium chloride, crystal 20 - 30 mesh
- Standard sulfate solution

C - Procedure

- Measure 100 ml sample or a suitable aliquot made up to 100 ml into a 250-ml erlenmeyer flask. Add 5 ml of conditioning agent and mix in the stirring apparatus. While the solution is being stirred, add a spoonful of barium chloride and stir for exactly 1 minute at a constant speed.
- Measure the optical density with the spectrophotometer at wavelength 420 mμ
- Estimate the sulfate concentration by comparing the optical density with a standard curve obtained by carrying out the same procedure with the standard solution.

APPENDIX IX

A - Sulfate Determination Accuracy

A test was made in order to evaluate the accuracy of the sulfate determination.

Five samples of the same solution were analyzed successively in different times.

The standard deviation, computed from the experiments is

$$S.D. = \pm 53 \text{ ppm}$$

for a solution that had an average sulfate concentration

$$S = 1336 \text{ ppm}$$

B - Flow Control Accuracy

A series of five runs with distilled water was carried out in order to measure the accuracy of the flow control at low flow rates.

The average flow rate was

$$F = 67.9 \text{ ml/hour}$$

that corresponds, for a continuous run, to a dilution ratio of $0.0565\ h^{-1}$.

The standard deviation was found to be:

$$S.D. = \pm 3.2 \text{ ml/hour}$$